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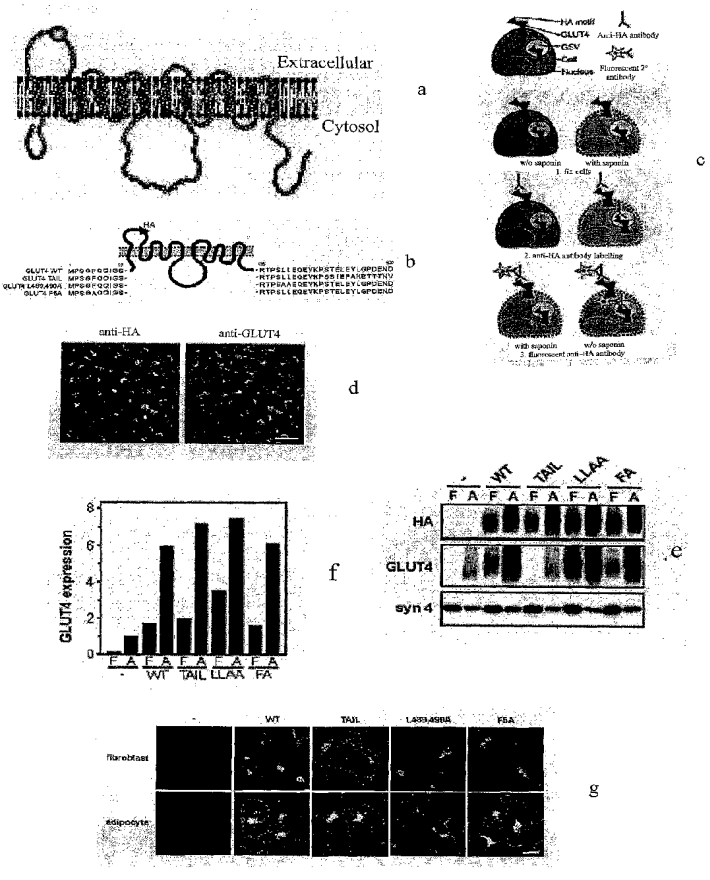

(57) Abstract: The present invention relates to a novel in vitro assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. The process of the invention is also useful for determining the level of recycling of a membrane transport protein. The present invention additionally provides a process for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.

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Novel translocation assay

Field of the invention

The present invention relates to a novel *in vitro* assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. In one embodiment, the present invention provides a method for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.

Background of the Invention

General

This specification contains nucleotide and amino acid sequence information prepared using PatentIn Version 3.1, presented herein after the claims. Each nucleotide sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, <210>3, etc). The length and type of sequence (DNA, protein (PRT), etc), and source organism for each nucleotide sequence, are indicated by information provided in the numeric indicator fields <211>, <212> and <213>, respectively. Nucleotide sequences referred to in the specification are defined by the term "SEQ ID NO:", followed by the sequence identifier (eg. SEQ ID NO: 1 refers to the sequence in the sequence listing designated as <400>1).

The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of elements or integers.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

Each embodiment described herein is to be applied *mutatis mutandis* to each and every other embodiment unless specifically stated otherwise.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

The present invention is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, peptide synthesis in solution, solid phase peptide synthesis, and immunology. Such procedures are described, for example, in the following texts that are incorporated by reference:

Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Second Edition (1989), whole of Vols I, II, and III;

DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL Press, Oxford, whole of text;

Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed., 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp1-22; Atkinson *et al.*, pp35-81; Sproat *et al.*, pp 83-115; and Wu *et al.*, pp 135-151;

Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins, eds., 1985) IRL Press, Oxford, whole of text;

Animal Cell Culture: Practical Approach, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970, whole of text;

Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text;

Perbal, B., A Practical Guide to Molecular Cloning (1984);

Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series;

J.F. Ramalho Ortigão, "The Chemistry of Peptide Synthesis" *In:* Knowledge database of Access to Virtual Laboratory website (Interactiva, Germany);

Sakakibara, D., Teichman, J., Lien, E. Land Fenichel, R.L. (1976). *Biochem. Biophys. Res. Commun.* **73** 336-342

Merrifield, R.B. (1963). *J. Am. Chem. Soc.* 85, 2149-2154.

Barany, G. and Merrifield, R.B. (1979) in *The Peptides* (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York.

Wünsch, E., ed. (1974) *Synthese von Peptiden in Houben-Weyls Metoden der Organischen Chemie* (Müler, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart.

Bodanszky, M. (1984) *Principles of Peptide Synthesis*, Springer-Verlag, Heidelberg.

Bodanszky, M. & Bodanszky, A. (1984) *The Practice of Peptide Synthesis, Springer-Verlag,* Heidelberg.

Bodanszky, M. (1985) *Int. J. Peptide Protein Res.* **25**, 449-474.

Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications).

Description of the related art

An important activity performed by any cell is the transport of materials across the plasma membrane. This activity is essential for the survival of all organisms, from simple unicellular organisms, e.g. bacteria, to complex multicellular organisms, e.g. humans. Not only does membrane transport facilitate the uptake of, for example, nutrients and ions, but also the excretion of waste products, and the secretion of signaling molecules..

The process of membrane transport itself is performed by a large class of proteins known as "transporters" "membrane transporters" "membrane transport proteins". A number of these proteins function by forming protein channels in the plasma membrane of a cell. This class of proteins includes a vast number of proteins that are related by their ability to transport other molecules across a cell membrane. It is hypothesized that the number of proteins involved in membrane transport constitute approximately 5% to 10% of known open reading frames in most sequenced genomes.

Membrane transport proteins are generally localized both intracellularly and within the plasma membrane. However, as the membrane-localized form is capable of transport activity, the amount of any membrane transport protein present in the plasma membrane limits the transport of substrates (both naturally-occurring substrates and small molecules) into and/or outside of the cell. Exemplary membrane transport proteins include the glucose-transporters (e.g. GLUT1, GLUT4), water transporters (e.g., aquaporins) and ion transporters that transport Cl^-, K^+, Na^+, Cu^{2+} or SO_4^{2-} ions, amongst others (e.g. cystic fibrosis transmembrane regulator (CFTR), pendrin, human ether-a-go-go (HERG)). As will be known to those skilled in the art, membrane transport proteins may function in the transport of multiple substrates for example, in the same direction (e.g., symport) across the plasma membrane or in the opposite direction (eg., antiport) across the plasma membrane.

Cells utilize a number of transport mechanisms, all of which are controlled by transport proteins.

Facilitated diffusion utilizes membrane protein channels to allow charged molecules (which otherwise could not diffuse across a plasma membrane) to freely move across a plasma membrane. For example, K+, Na+, and Cl- are transported across a plasma membrane by such membrane protein channels.

Facilitative transport molecules convey molecules, such as, for example, sugars down a concentration gradient, i.e. from a region of high concentration of that molecule to a region of low concentration, in a process that does not require energy.

In contrast, active transport requires the expenditure of energy to transport the molecule across the membrane. Similar to facilitated transport, active transport is limited by the number of membrane transport proteins present at the membrane.

Active, or coupled, membrane transporters transport substrates against a concentration gradient in a process that either requires energy expenditure or the use of another concentration gradient. For example, sodium dependent glucose transporters couple the transport of one molecule of glucose to two molecules of sodium. Sodium ions are transported down their concentration in a process that generates sufficient free energy to transport glucose against its concentration gradient allowing for a significant increase in the concentration of glucose in a cell.

As membrane transport proteins are involved in such a variety of functions that are essential to the survival of an organism, it is not surprising that several of these proteins have been found to be associated with disease in humans. For example, several forms of hearing loss in humans are associated with mutations in genes encoding transport proteins such as, for example, connexin 26, and pendrin, a proposed sulfate transporter. Defects in ion transporters are associated with a predisposition to cardiac arrhythmia, Menke's disease, Wilson's disease, familial generalized epilepsy, benign infantile epilepsy, spinocerebellar ataxia and familial hemiplegic migraine amongst many others.

Additionally, deficiency of the water channel protein aquaporin 2 hinders its translocation to the apical surface of the cell abolishing reabsorption of water from the collecting duct and resulting in nephrogenic diabetes insipidus.

Diabetes is associated with a dysfunctional glucose uptake into muscle and fat cells due to the impaired ability of insulin to stimulate glucose transporters.

In addition to mutations that directly affect the activity of a protein, any defect that inhibits the trafficking of the relevant membrane transport protein to the correct subcellular location has also been shown to be linked with human disease. For example, it has been suggested that the membrane transport protein GLUT4 is abnormally localized in type II diabetes (Bryant *et al, Nature Reviews Molecular Cell Biology, 3*, 267-277, 2002). In a normal cell GLUT4, which transports glucose across the plasma membrane, is thought to be almost entirely intracellular in the absence of insulin. Upon the addition of insulin, GLUT4 translocates to the plasma membrane.

However, in skeletal muscle cells from some type II diabetes mellitus subjects (Kelley *et al, J. Clin. Invest. 97*, 2705-2713, 1996) GLUT4 translocation has been shown to be drastically reduced. These results suggest impaired glucose transport as a consequence of impaired GLUT4 translocation may play a role in insulin resistance in type II diabetes.

The most common mutations in the cystic fibrosis transmembrane regulator (CFTR) gene (the ΔF508 mutation, Δ1507 mutation, K464M mutation, F508R mutation, and S5491 mutation, which account for approximately 70% of CF patients) have been suggested to cause abnormal localization of the CFTR protein to the endoplasmic reticulum, where it is subsequently degraded (Cheng *et al, Cell, 63(4)*, 827-834, 1990). Such mutant forms of the CFTR protein have been observed to be localized at the apical region of the cytosol of cells, rather than within the plasma membrane. As the CFTR protein is a chloride channel, the reduction in the amount of this channel in the membrane is associated with reduced movement of both sodium and water into the cell. The mislocalization of the CFTR protein has also been suggested as a possible causative factor in the reduced movement of sodium and water observed in the lungs and intestines of subjects suffering from cystic fibrosis.

In the case of cardiac arrhythmia, mutations have been found in the genes encoding the potassium channels, human ether-a-go-go-related gene (HERG), and KVLQT1. The HERG protein is the pore-forming subunit of the cardiac rapidly activating delayed rectifier potassium channel. In both cases, mutations in the gene encoding each protein are associated with a reduction with trafficking of the protein and, as a consequence, a reduction in the amount of the protein being integrated into the plasma membrane. As a result, cardiac cells expressing the mutant protein show reduced amplitude and altered voltage dependence of activation (Zhou *et al, J. Biol. Chem., 274(44)*, 31123-31126, 1999).

Mutations in various other membrane transport proteins have also been suggested to cause a number of disorders due to altered or incorrect trafficking/translocation of the mutant protein, for example, glucose-galactose malabsorption, changes in cholesterol homeostasis, and defects in the multi-drug transporter P-glycoprotein.

As membrane transport proteins are involved in several essential cellular processes, and mutations affecting the function and/or localization of these proteins are involved in the

etiology of certain human diseases, there is a clear need in the art for methods of detecting mutations in these proteins and/or modulatory agents that affect their subcellular localization and/or turnover/recycling.

Known methods of determining the activity of a membrane transport protein generally involve the mere measurement of the movement of a specific substrate across a lipid bilayer, such as that found at the membrane of a cell. These methods are imprecise, as any redundancy in the transport process of interest, e.g. if a cell expresses multiple proteins that transport the same molecule, may mask or reduce the effect of a mutation of one of the constituents (i.e. transport proteins) of the process. For example, there are at least 12 hexose transporters encoded by the genes in the human genome and most mammalian cell types express more than one member of this family.

Alternatively, plasma membranes are isolated and low density microsomal fractions prepared. The membrane transport proteins are then photolabeled (e.g. bis-mannose photolabeling of GLUT4 located on the cell surface), and subsequently immunoprecipitated e.g. as described in Homan *et al., J. Biol. Chem. 26:5* 18172-18179 (1990).

Alternatively, plasma membrane sheets are prepared for use in microscopic analysis essentially as described in Cushman and Wardzala., *J Biol Chem. 255:*4758-4762 (1980), or by isolation of plasma membrane sheets or lawns for use in microscopic analysis as described in Robinson, *et al., J Cell Biol. 117*:1181-1196 (1992).

These assays are both laborious and subject to inter-assay variability, and furthermore, are only semi-quantitative. Accordingly, the quantitative nature of these assays is limited. Furthermore, these assays are not readily adapted to high-throughput analysis, for example, for screening compounds that modulate translocation of a membrane transport protein.

Accordingly, there is a clear need in the art for a straightforward, reproducible method for the detection and estimation of the level of a membrane transport protein translocated to the plasma membrane. Preferred assays will not require sub-cellular fractionation or multiple labeling. Preferred assays will also be useful for determining mutations and/or agents that affect translocation of the membrane transport protein, for example, in a high-throughput assay.

Summary of the Invention

In work leading up to the present invention, the inventors sought to develop an assay that detects the level of a membrane transport protein incorporated into the plasma membrane of a cell compared to the total level of said membrane transport protein within the cell. Furthermore, the inventors sought to use this assay to determine the level of trafficking and/or turnover of the membrane transport protein at the plasma membrane.

For example, the present inventors have developed an assay useful for determining the level of GLUT4 translocation in a cell. The assay uses a GLUT4 protein that is labeled with a tag or marker that facilitates detection of the GLUT4. Preferably, the tag or marker is located within an extracellular domain of the GLUT4 protein. The location of the tag or marker facilitates detection of the GLUT4 protein at the plasma membrane of an intact cell. By determining the level of tagged/marked GLUT4 protein at the plasma membrane of a cell relative to the level of tagged/marked GLUT4 in the cell, the level of GLUT4 translocation is determined.

The present inventors have additionally shown that the process of the present invention is amenable to performance in 96-well and 384-well formats. Accordingly, this assay provides a high throughput screen to determine a modulator of translocation of a membrane transport protein. Such a modulator represents a candidate therapeutic for the treatment of a disease associated with translocation (e.g. aberrant translocation) of a membrane transport protein.

Furthermore, the present inventors have developed a model of insulin resistance observed in subjects suffering from type-II diabetes. This assay provides the basis for a screen to determine a candidate compound for the treatment of insulin resistance e.g. that associated with type-II diabetes.

The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:

(a) determining the level of a membrane transport protein at the plasma membrane of the cell using a method comprising:

(i) contacting the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein at the plasma membrane of the cell; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein; and

(c) comparing the level of ligand determined at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the level of the membrane transport protein inside the cell.

For example, the membrane transport protein is a glucose transport (GLUT) protein.

In an example, the membrane transport protein is GLUT4, e.g., the GLUT4 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.

In another example, the membrane transport protein is GLUT1 e.g., the GLUT1 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 12.

In yet another example, the membrane transport protein is a mutant membrane transport protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.

For example, the mutant membrane transport protein is a mutant glucose transport (GLUT) protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.

For instance, the reduced rate of recycling or transporter internalization of the mutant membrane transport protein increases the level of the mutant membrane transport

protein at the plasma membrane of a cell compared to the level of a wild-type form of the membrane transport protein.

In an example, the mutant GLUT protein is a mutant GLUT4 protein, e.g., the mutant GLUT4 protein comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

For example, the membrane transport protein is labeled to facilitate binding of the ligand to the membrane transport protein.

In an example, the label comprises one or more copies of a peptide, polypeptide or protein that is heterologous to the membrane transport protein. For example, the label comprises one or more copies of a peptide, polypeptide or protein selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β-galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-shifted green fluorescent protein (SEQ ID NO: 29), cyan fluorescent protein (SEQ ID NO: 30) , biotin, strepavidin, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 20, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 21, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 31 and mixtures thereof.

In one exemplified form of the invention, the label comprises the amino acid sequence set forth in SEQ ID NO: 8.

For example, the label is positioned within an extracellular domain of the membrane transport protein, e.g., the label is positioned within the first extracellular domain of a GLUT protein or a mutant thereof.

For example, the labeled membrane transport protein is a GLUT4 protein or a mutant GLUT4 protein that comprises an amino acid sequence at least 80% identical to an

amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

In another example, the labeled membrane transport protein is a GLUT1 protein that comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 13.

In an example of the invention, the cell is a eukaryotic cell, for example, the cell is a mammalian cell, e.g., a cell selected from the group consisting of a 3T3-L1 fibroblast cell, a 3T3-L1 adipocyte cell and a C2C12 cell.

In an example, the ligand capable of binding to the membrane transport protein is an antibody. For example, the antibody is a monoclonal antibody, e.g., an anti-HA tag antibody.

For example, the antibody is labeled with a detectable marker selected from the group consisting of an enzyme label, a radiolabel and a fluorescent label, e.g., the antibody is labeled with a fluorescent label.

In an example, the plasma membrane is permeablilized or disrupted by contacting the plasma membrane with an agent that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur. For example, the agent that permeabilizes or disrupts a membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β-D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α-hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof, e.g., the agent that permeabilizes or disrupts the membrane is saponin.

In an example of the invention, the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds to the ligand for a time and under conditions sufficient for an antibody-antigen complex to form and determining the level of the complex wherein the level of the complex indicates the level of the ligand bound to the membrane transport protein.

For example, the level of the ligand bound to the membrane transport protein is determined using an assay selected from the group consisting of immunfluorescence, immunohistochemistry, and an immunosorbent assay, e.g., the level of the ligand bound to the membrane transport protein is determined using a fluorescence linked immunosorbent assay.

In one example, the process of the invention additionally comprises providing the cell expressing the membrane transport protein. For example, providing the cell expressing the membrane protein comprises transforming or transfecting the cell with an expression construct that encodes the membrane protein.

In an example, the process additionally comprises fixing the cell. For example, the cell is fixed prior to or at the same time as permeabilizing or disrupting the plasma membrane of the cell.

In an example, the cell is fixed with a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol and glutaraldehyde, e.g., the cell is fixed with formaldehyde.

In another example, the present invention additionally comprises inducing translocation of the membrane transport protein to the plasma membrane. For example, inducing translocation of the membrane transport protein to the plasma membrane comprises contacting the cell with an amount of one or more peptides, polypeptides, proteins or compounds sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur.

For instance the cell is contacted with sucrose and/or insulin, e.g., the cell is contacted with sucrose and/or insulin in the presence of serum.

In another example, the process additionally comprises inducing resistance to translocation of the membrane transport protein in the cell. For example, the membrane transport is a GLUT protein or a mutant GLUT protein and wherein inducing resistance to translocation of the membrane transport protein in the cell comprises contacting the cell with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient for resistance to insulin induced translocation to occur.

For example, the cell is contacted with insulin in the absence of serum, e.g., the cell is contacted with insulin for between about 24 hours and about 48 hours.

The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said process comprising:

(a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:

(i) contacting a cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) determining the level of the membrane transport protein within another cell using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;

(iii) determining the level of ligand bound to the membrane transport protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.

For example, the cells are isogenic or from the same cell line.

For instance, the cells are cultured under substantially similar conditions.

In an example, the level of the membrane transport protein at the plasma membrane of the cell and the level of membrane transport protein within the cell are each determined in a plurality of cells.

For example, the process of the invention additionally comprises normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of ligand bound to the membrane transport protein is determined.

For example, the number of cells is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a cell or component thereof for a time and under conditions sufficient for binding of the antibody or ligand to the cell or component thereof and determining the level of antibody bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of the number of cells, e.g., the ligand is wheat germ agglutinin.

The present invention additionally provides a process for determining the level of a labeled GLUT4 protein or labeled mutant GLUT4 protein translocated to the plasma membrane of a cell, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
  (i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and
  (ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(b) determining the level of membrane transport protein within another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:
  (i) permeabilizing or disrupting the other cell;
  (ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;
  (iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

The present invention additionally provides a process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell, wherein the cells are contacted with insulin in the absence of serum and wherein the cells are contacted with insulin for a period of time from about 24 hours to about 48 hours;

(b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell (a) using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(c) determining the level of labeled GLUT4 protein or labeled mutant GLUT4 protein in another cell (a) using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the

plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

The present invention additionally provides a process for determining the level of recycling of a membrane transport protein in a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to determine the level of recycling of the membrane transport protein in the cell.

The present invention additionally provides a process for determining a change in the level of recycling of a membrane transport in a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b),

wherein a change in the level of the membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

The present invention additionally provides a process for determining a mutation in a nucleic acid encoding a mutant membrane transport protein, wherein said mutation modulates translocation of said membrane transport protein, said method comprising:

(i) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process of the invention; and

(ii) determining the level of the wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention,

wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that modulates the level of level of translocation of the membrane transport protein to the plasma membrane.

The present invention additionally provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

(a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention, wherein a difference in the level of the membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(e) optionally, providing, the candidate agent.

The present invention further provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

(a) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4

translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(e) optionally, providing, the candidate agent.

For example, the insulin resistance is associated with diabetes, e.g., the diabetes is type II diabetes.

The present invention additionally provides a process for manufacturing a medicament for the treatment of insulin resistance comprising:

(a) determining a candidate compound for the treatment of insulin resistance using a process comprising:

(i) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

(b) optionally, isolating the candidate agent;

(c) optionally, providing the name or structure of the candidate agent;
(d) optionally, providing the candidate agent; and
(e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

Brief description of the figures

Figure 1A is a schematic representation of a recombinant GLUT4 protein that is labeled with a HA epitope. Note that when expressed in a cell the HA epitope is within the first extracellular domain of the protein. This location of the HA epitope facilitates detection of the GLUT4 protein when translocated to the plasma membrane without disrupting said plasma membrane.

Figure 1B is a schematic representation showing the various forms of GLUT4 used in the analysis of translocation of GLUT4 to the plasma membrane. WT represents the wild-type form of GLUT4 (SEQ ID NO: 1) TAIL represents a mutant form of GLUT4 in which the residues at the C-terminus of GLUT4 have been mutated (SEQ ID NO: 5); L489,490A represents a mutant form of GLUT4 in which a di-leucine motif at the C-terminal end of GLUT4 has been mutated to a di-Alanine motif (SEQ ID NO: 6); and F5A represents a mutant form of GLUT4 in which the phenylalanine at amino acid number 5 of GLUT4 has been mutated to Alanine (SEQ ID NO: 7), wherein each of these proteins have been labeled with a HA epitope tag (SEQ ID NO: 18) in an intracellular domain, for example, the sequence of a WT, GLUT4 labeled with an HA epitope tag is represented by SEQ ID NO: 3.

Figure 1C is a schematic representation of one example of the method of detecting the amount of GLUT4 that has translocated to the plasma membrane. The left hand side of the figure shows a cell that is stained to determine the amount of GLUT4 that has translocated to the membrane. Recombinant GLUT4 labeled with a HA epitope is expressed in the cell; the cell is then fixed and the GLUT4 that has translocated to the plasma membrane is detected with an anti-HA antibody; the cell is then permeabilized with saponin and the anti-HA antibody detected with a fluorescent secondary antibody. The right hand side of the figure shows a cell that is used to determine the total amount of GLUT4 in a cell. Recombinant GLUT4 labeled with a HA epitope is expressed in the cell; the cell is then fixed; and permeabilized with saponin. The HA epitope is then detected with an anti-HA antibody, which is now able to enter the cell. The anti-HA epitope is then detected with a fluorescent secondary antibody. Comparing the results

obtained from the two cells shows the amount of GLUT4 that has translocated to the plasma membrane as a function of total GLUT4.

Figure 1D is a copy of a photographic representation showing 3T3-L1 adipocytes expressing HA-GLUT4 WT immunolabeled with an anti-HA or anti-GLUT4 for the detection of HA-GLUT4 or total GLUT4 content respectively.

Figure 1E is a copy of a photographic representation showing an immunoblot on which cell extracts from 3T3-L1 fibroblasts (F) or 3T3-L1 adipocytes (A) expressing the indicated HA-tagged GLUT4 protein were analyzed using the indicated antibody (left hand side).

Figure 1F is a graphical representation showing the level of expression of each of the HA-tagged GLUT4 proteins shown in Figure 1C

Figure 1G is a copy of a photographic representation of various cells used to analyze the translocation of GLUT4. The top row of cells are 3T3-L1 fibroblasts and the bottom row 3T3-L1 adipocytes. From left to right the cells were not transduced (i.e. do not express a tagged GLUT4); were transduced with a tagged WT, GLUT4; were transduced with a tagged TAIL mutant GLUT4; were transduced with a tagged L489,490A mutant GLUT4; or were transduced with a tagged F5A mutant GLUT4.

Figure 2A is a graphical representation of the effect of insulin that do not express HA-tagged GLUT4. The amount of fluorescence detected using the anti-HA antibody (HA) was the same as that detected with a non-relevant (NR) antibody, indicating that the anti-HA antibody does not non-specifically bind a protein in the cell.

Figure 2B is a graphical representation of the amount of HA tagged GLUT4 detected at the plasma membrane of 3T3-L1 adipocytes incubated in the presence of 200 nM insulin. Over time, the amount of HA-tagged GLUT4 (squares) detected at the plasma membrane increased, while the amount of the non-relevant protein (triangles) remained constant. This indicates that insulin induces GLUT4 translocation to the plasma membrane.

Figure 2C is a graphical representation of the percentage of total GLUT4 in a cell that has translocated the plasma membrane in the presence of 200 nM insulin. Using the

method described herein the amount of HA tagged GLUT4 that was translocated to the plasma membrane in the presence of insulin was determined relative to the total HA-tagged GLUT4 in a cell.

Figure 2D is a graphical representation of the percentage of total GLUT4 in a cell that has translocated to the plasma membrane in the presence of various concentrations of insulin. Using the method described herein the effect of insulin concentration on the amount of HA-tagged GLUT4 translocation to the plasma membrane relative to the total HA-tagged GLUT4 was determined (triangle). In the presence of wortmannin (squares) insulin induced translocation of GLUT4 was almost totally abrogated.

Figure 3A is a graphical representation showing the amount of a HA-tagged form of GLUT4 (from left to right: WT; TAIL; L489; 490A; and F5A) detected at the plasma membrane of 3T3-L1 fibroblasts at relative to the total HA-tagged form of GLUT4. Clearly GLUT4 translocation is induced by insulin in fibroblasts.

Figure 3B is a graphical representation showing the percentage of a HA-tagged form of GLUT4 (from left to right: WT; TAIL; L489; 490A; and F5A) at the plasma membrane of 3T3-L1 adipocytes in the presence of 200 nM insulin. Interestingly, the L489; L490A and F5A mutants, which are believed to be impaired in their internalization/recycling, show an increase in adipocytes compared with fibroblasts (Figure 3A).

Figure 4 is a graphical representation showing the internalization kinetics of HA-GLUT4 in 3T3-L1 adipocytes. Adipocytes expressing the indicated GLUT4 molecule were incubated for 20 min with 200 nM insulin at 37°C and for 1 h with anti-HA antibody on ice. Excess antibody was washed away, and cells were incubated for the indicated periods at 37°C in the presence of either 100 nM wortmannin, to measure GLUT4 internalization in the basal state, or 200 nM insulin. Cells were exposed to fixative and incubated with fluorescent secondary antibody in the absence of permeabilizing agent to allow measurement of the time-dependent disappearance of anti-HA-labeled GLUT4 from the cell surface.

Figure 5A is a copy of a photographic representation showing the subcellular localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM

insulin and subsequently for 2 hours without insulin and then 20 minutes without insulin.

Figure 5B is a copy of a photographic representation showing the subcellular localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM insulin and subsequently for 2 hours with insulin and then 20 minutes without insulin.

Figure 5C is a copy of a photographic representation showing the subcellular localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM insulin and anti-HA antibody and subsequently for 2 hours without insulin and anti-HA antibody and then 20 minutes without insulin.

Figure 5D is a copy of a photographic representation showing the subcellular localization of HA-tagged GLUT4 in adipocytes incubated for 2 hours with 200 nM insulin and anti-HA antibody and subsequently for 2 hours without insulin and anti-HA antibody and then 20 minutes with insulin.

Figure 5E shows graphical representations showing levels of antibody uptake in fibroblasts or adipocytes as indicated at the left hand-side of the figure expressing the indicated HA-GLUT4 protein. Cells were incubated with (squares) or without (triangles) 200nM insulin for 20 min, after which anti-HA antibody was added. Cells were incubated for up to 180 minutes, fixed permeabilized and incubated with a fluorescently labeled secondary antibody. The level of anti-HA antibody taken up by the cells is expressed as a percentage of total post-fixation anti-HA labeling.

Figure 6A is a graphical representation demonstrating the existence of a non-recycling pool of HA-GLUT4 WT in a cell. Cells were incubated in the presence of insulin for an extended period of time (180min) and the level of HA-GLUT4 at the plasma membrane relative to the total level detected in the cell was determined.

Figure 6B is a graphical representation showing the level of HA-GLUT4 in the cells used to determine the level of HA-GLUT4 in the cell (Figure 6A) following an additional incubation with fixative.

Figure 6C is a graphical representation showing the level of HA-GLUT4 detected at the plasma membrane of cells in which the level of HA-GLUT4 at the plasma membrane

was previously determined (Figure 6A) following an additional incubation with an anti-HA antibody (and detection of the level of bound anti-HA antibody).

Figure 6D is a graphical representation showing the level of of HA-GLUT4 detected within cells previously fixed and permeabilized following an additional incubation with an anti-HA antibody (and detection of the level of bound anti-HA antibody).

Figure 6E is a graphical representation showing the relative level (percentage of total) level of HA-GLUT4 WT detected at the plasma membrane of a cell using various concentrations of anti-HA antibody.

Figure 6F is a graphical representation showing the relative level (percentage of total) of HA-GLUT4 WT detected at the plasma membrane of a cell following a 2 hour incubation in the presence of cycloheximide.

Figure 6G is a graphical representation showing the effect of endosomal pH on the binding of the anti-HA antibody to HA-GLUT4. Cells were incubated for 30 min at 37□C in hypertonic medium (0.45 M sucrose, pH 7.4), on ice with antibody in the same medium, and at 37□C in hypertonic buffer at pH 7.4 or pH 5.5 in the absence of antibody. Release of antibody from the PM at neutral or endosomal pH was determined by incubating fixed non-permeabilized cells with fluorescent secondary antibody.

Figure 6H is a graphical representation showing the effect of incubating a cell in the presence of insulin for an extended period of time. Cells were incubated in the presence of 200nM insulin for up to 3 hours and the relative level (percentage of total) of HA-GLUT4 at the plasma membrane determined.

Figure 7 shows graphical and photographic representations showing GLUT4 recycling during the differentiation of 3T3-L1 fibroblasts into adipocytes. FIG. 5. Cells were analyzed at different stages during differentiation as indicated. After incubation for 18 h in medium containing fetal bovine serum and for 2 h in the absence of serum, the cells were incubated in the continuous presence of anti-HA antibody as described for Fig. 4. Parallel cultures were incubated similarly but analyzed by immunofluorescence confocal microscopy (left microscopy panels). Non-infected cells were analyzed for endogenous GLUT4 and lipid droplet content during differentiation (right microscopy panels). Bottom

right microscopy panels show Z section image of the cells. White dotted lines mark the contours of the cells.

Figure 8A is a graphical representation showing a correlation between insulin concentration and the size of the non-recycling GLUT4 pool in 3T3-L1 adipocytes. 3T3-L1 adipocytes expressing HA-GLUT WT or HA-GLUT TRAIL were incubated at 37°C with anti-HA antibody and the indicated concentration of insulin and the level of cell associated HA antibody was determined.

Figure 8B is a graphical representation showing 3T3-L1 adipocytes expressing HA-GLUT4 WT or HA-GLUT4 TAIL that were incubated for 20 min at 37oC with 0.032, 0.24, 3.2, 15 or 200 nM insulin and amounts of GLUT4 at the PM were determined and expressed as percentage of maximal insulin-induced GLUT4 translocation.

Figure 8C is a copy of a photographic representation showing HA-GLUT4-expressing 3T3-L1 adipocytes incubated for 3 h with anti-HA antibody and the indicated concentrations of insulin. Cells were fixed, permeabilized, incubated with fluorescent secondary antibody and analyzed by confocal immunofluorescence microscopy.

Figure 9 is a graphical representation showing the translocation of HA-GLUT4 in 3T3-L1 adipocytes grown and differentiated in a 384-well plate compared to cells grown and differentiated in a Petri dish and transferred to a 384-well plate. Axes are time of insulin exposure (min, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).

Figure 10 is a graphical representation showing the effect of amino acid concentration on the level of HA-GLUT4 translocated to the plasma membrane of a cell. HA-GLUT4 expressing adipocytes were serum starved for 2 hours in Krebs Ringer Phosphate buffer or in the same buffer supplemented with amino acid concentrations used in Dulbecco's modified eagle medium of Gibco (2x amino acids) or with half of the amino acid concentration (1x amino acids) as indicated. Axes are time of insulin exposure (min, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).

Figure 11 is a graphical representation showing the effect of insulin and sucrose on HA-GLUT4 translocation. 3T3-L1 adipocytes expressing HA-GLUT4 WT were serum

starved for 2 hours at 37°C. Following 20 minutes of acute insulin stimulation with 200nM, cells were incubated for additional 2 hours in serum free medium supplemented with 0.2% BSA and 0.3 or 0.6M sucrose as indicated. After post-fixation anti-HA immunolabeling the amount of cell surface HA-GLUT4 levels was determined. Axes are insulin concentration (nM, X-axis) and percentage of total HA-GLUT4 detected at the plasma membrane (Y-axis).

Figure 12A is a graphical representation showing the induction of insulin resistance in 3T3-L1 adipocytes. 3T3-L1 adipocytes retrovirally infected with HA-GLUT4 were incubated 24 hours or 48 hours either with 600nM insulin or with medium alone. After this chronic insulin stimulation for the indicated periods of time, cells were washed and 200 nM insulin added for additional 10 or 30 minutes and cell surface levels of HA-GLUT4 were measured using the fluorescence based assay. Treatment groups are indicated. Y axis shows the percentage of total HA-GLUT4 detected at the plasma membrane.

Figure 12B is a graphical representation showing the induction of insulin resistance in 3T3-L1 adipocytes expressing a mutant GLUT4. 3T3-L1 adipocytes retrovirally infected with HA-GLUT4 TAIL mutant were incubated 24 hours or 48 hours either with 600nM insulin or with medium alone. After this chronic insulin stimulation for the indicated periods of time, cells were washed and 200 nM insulin added for additional 10 or 30 minutes and cell surface levels of HA-GLUT4 TAIL were measured using the fluorescence based assay. Treatment groups are indicated. Y axis shows the percentage of total HA-GLUT4 detected at the plasma membrane.

Figure 13 is a graphical representation showing the effect of wortmannin on acute and chronic insulin induced GLUT4 translocation. HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 96 well plates, incubated for 2 hours or overnight in medium supplemented with 10% fetal calf serum or no serum. 200nM insulin in case of acute stimulation and 600nM insulin in case of chronic stimulation were used (as indicated). Following overnight stimulation cells were washed and 200nM fresh insulin was added for 10 or 30 min. Both medium conditions were tested in the presence and absence of 100nM wortmannin. Y axis shows the percentage of total HA-GLUT4 detected at the plasma membrane.

Detailed description of the preferred embodiments

The present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:

(a) determining the level of a membrane transport protein at the plasma membrane using a method comprising:

(i) contacting the membrane transport protein with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein within the cell; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the level of the membrane transport protein inside the cell.

For example, a ligand of a membrane transport protein that binds to an extracellular domain of the membrane transport protein is, for example, an antibody. Antibodies that bind an extracellular domain of a membrane protein are known in the art. For example, monoclonal antibody mAb5 or mAb263 that specifically bind an extracellular region of the growth hormone receptor protein (available from AGEN Limited, Acacia Ridge, Queensland, Australia). A polyclonal antibody that bind to an extracellular domain of GLUT2 is available from Alpha Diagnostics International Inc., San Antonio, TX, USA. An antibody that binds to an extracellular domain of GLUT1 is described in Carbó *et al., Clinical and Experimental Pharmacology and Physiology 30*: 64, 2003. Alternatively, the antibody or ligand is produced by a method known in the art and/or described herein.

Membrane transport proteins

As used herein, the term "membrane transport protein" shall be taken to mean a peptide, polypeptide or protein that catalyzes the movement of a molecule across a

membrane, whether this movement is by diffusion (simple or facilitated) or active transport. Membrane transport proteins in the present context exist as intracellular proteins and are capable of being membrane-localized. Such a protein may be, for example, a channel, a transporter, an ATP pump, a symporter or an antiporter. The term "membrane transport protein" shall be taken to include mutant forms of a membrane transport protein (for example, a mutant form of a membrane transport protein capable of translocating to the plasma membrane of a cell) and/or a labeled membrane transport protein. For example, a labeled membrane transport protein described herein.

For example, a membrane transport protein useful in performance of the invention is a protein from a family of proteins selected from the group consisting of amino acid/auxin permease (AAAP) family, amino acid-polyamine-organocation (APC) family, cation-chloride cotransporter (CCC) family, hydroxy/aromatic amino acid permease (HAAAP) family, bile acid:NA^+ symporter (BASS) family, arsenical resistance-3 (ARC3) family, monovalent cation:proteon antiporter-1 (CPA1) family, monovalent cation:proton antiporter-2 (CPA2) family, Na^+-transporting carboxylic acid decarboxylase (NaT-DC) family, citrate-Mg^{2+}:H^+ (MitM) citrate-Ca^{2+}:H^+ (CitH) symporter (CitMHS) family, C_4-dicarboxylate uptake (Dcu) family, lactate permease (LctP) family, NhaB Na^+:H^+ antiporter (NhaB) family, NhaC Na^+:H^+ antiporter (NhaC) family, arsenite-antimonite (ArsB) efflux family, divalent anion:Na^+ symporter (DASS) family, tripartite ATP-independent periplasmic transporter (TRAP-T) family, C_4-dicarboxylate uptake C (DcuC) family, NhaD Na^+:H^+ antiporter (NhaD) family, p-aminobenzyol-glutamate transporter (AbgT) family, gluconate:H^+ symporter (GntP) family, L-lysine exporter (LysE) family, major facilitator superfamily (MFS), proton-dependent oligopeptide transporter (POT) family, organo-anion transporter (OAT) family, folate-biopterin transporter (FBT) family, PTS galactilol (Gat) family, PTS L-ascorbate (L-Asc) family, PTS glucose-glucoside (Glc) family, PTS fructose-mannitol (Fru) family, voltage-gated ion channel (VIC) family, glutamate gated ion channel (GIC) family of neurotransmitter receptors, animal inward rectifier K^+ channel (RIR-CaC) family, ryanodine-inositol 1, 4, 5-triphosphate receptor Ca^{2+} channel (RIR-CaC) family and K^+ transporter (Trk) family. Information concerning the structure and/or function of a membrane transport protein (e.g., a membrane transport protein from a family described *supra*) is found in, for example, the Transport Classification Database available from University of California, San Diego, La Jolla, Ca, USA.

For example, the membrane transport protein is a human membrane transport protein. For example, a human membrane transport protein selected from the group consisting of a human annexin, a human ATP-binding cassette transporter, a human ATPase, a human calcium channel, a human potassium channel, a human sodium channel and a human solute carrier.

For example, the membrane transport protein is a protein that translocates to a plasma membrane of a cell under normal physiological conditions, or following stimulation by a condition or agent, such as, for example, glucose or insulin. Preferably the membrane transport protein is, for example, an ABC transporter protein, a P class ATP pump, a F class ATP pump, a V class ATP pump, a Cl^- channel, a H^+ channel and Ca^{++} channel, a K^+ channel, an uniporter a symporter or an antiporter. For example, the membrane transport protein is a membrane transport protein selected from the group consisting of ABC1, ABCA2, ABCA3, ABCR, ABCA5, ABCA6, ABCA7, ABCA8, ABCA9, ABCA10, ABCA12, ABCA13, PGY1, TAP1, TAP2, PGY3, ABCB5, ABCB6, ABC7, M-ABC1, ABCB9, ABCB10, BSEP, MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, CFTR, SUR1, SUR2, ABCC10, ABCC11, ABCC12, ABCC13, ALD, ALDL1, ABCD2, PXMP1, PXMP1L, RNASELI, ABC50, ABCF2, ABCF3, ABCG1, ABCG2, ABCG4, ABCG5, ABCG8, KCNA1, CACNL1A4, KCNQ2, KCNQ3, SCN1B, CHRNA4, GLRA1, KCNE1, KCNQ4, SCN4A, CACNL1A3, CLCN1, CNCN1, RYR1, CHRNA1, KCNQ1, HERG, SCN5A, KCNE1, SCN5A, KCNE1, GLUT1, GLUT2, GLUT3, GLUT4, GLUT5, GLUT6, GLUT7, GLUT8, GLUT9, GLUT10, GLUT11, GLUT12, HMIT and GLUT14.

As used herein, the nomenclature for GLUT proteins and HMIT is described by Joost *et al, 2001, Am. J. Physiol. Endocrinol. Metab. 282*: E974-E976, 2002.

In an example of the invention, the membrane transport protein is a glucose transport protein or a facilitated glucose transport protein (GLUT). As used herein the term "glucose transport protein" or "facilitated glucose transport protein" or "GLUT" shall be taken to mean a member of the SCLC2A family of solute carrier proteins. Individual member of this family have similar predicted secondary structures with 12 transmembrane domains. Both N and C-termini are predicted to be cytoplasmic. There is a large extracellular domain between transmembrane region 1 and transmembrane region 2 and a large cytoplasmic domain between transmembrane region 6 and transmembrane region 7.

GLUT isoforms differ in their tissue expression, substrate specificity and kinetic characteristics. Table 1 outlines many of the characteristics of GLUT isoforms.

Table 1: GLUT isoforms

GLUT Isoform	Characteristics
GLUT1	mediates glucose transport into red cells, and throughout the blood brain barrier. It is ubiquitously expressed and transports glucose in most cells
GLUT2	provides glucose to the liver and pancreatic cells
GLUT3	the main glucose transporter in neurons
GLUT4	primarily expressed in muscle and adipose tissue and regulated by insulin
GLUT5	transports fructose in intestine and testis
GLUT6	highly expressed in brain, spleen, and leukocytes.
GLUT8	High levels are found in adult testis and placenta
GLUT9	expressed in kidney, liver, placenta, lung, blood leukocytes, heart, and skeletal muscle
GLUT10	widely expressed with highest levels in liver and pancreas
GLUT11	expressed in heart and skeletal muscle
GLUT12	expressed in skeletal muscle, adipose tissue, and small intestine
GLUT13	(aka. H+ myo-inositol transporter, HMIT) predominantly expressed in brain

For example, the process of the invention is performed with a GLUT protein selected from the group consisting of a GLUT1 protein, a GLUT2 protein, a GLUT3 protein, a GLUT4 protein, a GLUT5 protein, a GLUT6 protein, a GLUT7 protein, a GLUT8 protein, a GLUT9 protein, a GLUT10 protein, a GLUT11 protein, a GLUT12 protein, a GLUT13 (HMIT) protein, a GLUT14 protein.

As used herein, the term "GLUT1 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 12. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 12.

In one example, the GLUT1 protein is a human GLUT1 protein.

Alternatively, or in addition, a GLUT 1 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 11. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 11.

As used herein, the term "GLUT2 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 38. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 38.

In one example, the GLUT2 protein is a human GLUT2 protein.

Alternatively, or in addition, a GLUT2 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 37. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 37.

As used herein, the term "GLUT3 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 40. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 40.

In one example, the GLUT3 protein is a human GLUT3 protein.

Alternatively, or in addition, a GLUT3 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 39. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at

least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 39.

As used herein, the term "GLUT4 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 2. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 2.

In one example, the GLUT4 protein is a human GLUT4 protein.

Alternatively, or in addition, a GLUT 4 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 1. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 1.

As used herein, the term "GLUT5 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 2. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 42.

In one example, the GLUT5 protein is a human GLUT5 protein.

Alternatively, or in addition, a GLUT5 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 41. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 41.

As used herein, the term "GLUT6 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 44. For example, the protein comprises an amino acid sequence at least

about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 44.

In one example, the GLUT6 protein is a human GLUT6 protein.

Alternatively, or in addition, a GLUT6 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 43. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 43.

As used herein, the term "GLUT7 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 46. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 46.

In one example, the GLUT7 protein is a human GLUT7 protein.

Alternatively, or in addition, a GLUT7 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 45. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 45.

As used herein, the term "GLUT8 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 48. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 48.

In one example, the GLUT8 protein is a human GLUT8 protein.

Alternatively, or in addition, a GLUT8 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 47. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 4.

As used herein, the term "GLUT9 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 50. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 50.

In one example, the GLUT9 protein is a human GLUT9 protein.

Alternatively, or in addition, a GLUT9 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 49. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 49.

As used herein, the term "GLUT10 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 52. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 52.

In one example, the GLUT10 protein is a human GLUT10 protein.

Alternatively, or in addition, a GLUT10 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 51. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 51.

As used herein, the term "GLUT11 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 54. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 54.

In one example, the GLUT11 protein is a human GLUT11 protein.

Alternatively, or in addition, a GLUT11 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 53. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 53.

As used herein, the term "GLUT12 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 56. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 56.

In one example, the GLUT12 protein is a human GLUT12 protein.

Alternatively, or in addition, a GLUT12 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 55. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 55.

As used herein, the term "GLUT13 protein" or "HMIT" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 57. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least

about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 57.

In one example, the GLUT13 or HMIT protein is a human GLUT13 or HMIT protein.

Alternatively, or in addition, a GLUT13 or HMIT protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 56. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 56.

As used herein, the term "GLUT14 protein" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 59. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 59.

In one example, the GLUT14 protein is a human GLUT14 protein.

Alternatively, or in addition, a GLUT14 protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 58. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 58.

In an exemplified form of the invention, the membrane transport protein is a GLUT4 transport protein or a GLUT1 transport protein.

In determining whether or not two amino acid sequences fall within the defined percentage identity limits *supra*, those skilled in the art will be aware that it is possible to conduct a side-by-side comparison of the amino acid sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical residues depending upon the algorithm used to perform the alignment. In the present context, references to percentage identities and similarities between two or more amino

acid sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. In particular, amino acid identities and similarities are calculated using software of the Computer Genetics Group, Inc., University Research Park, Maddison, Wisconsin, United States of America, e.g., using the GAP program of Devereaux *et al., Nucl. Acids Res. 12*, 387-395, 1984, which utilizes the algorithm of Needleman and Wunsch, *J. Mol. Biol. 48*, 443-453, 1970. Alternatively, the CLUSTAL W algorithm of Thompson *et al., Nucl. Acids Res. 22,* 4673-4680, 1994, is used to obtain an alignment of multiple sequences, wherein it is necessary or desirable to maximize the number of identical/similar residues and to minimize the number and/or length of sequence gaps in the alignment.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul *et al. J. Mol. Biol. 215*: 403-410, 1990), which is available from several sources, including the NCBI, Bethesda, Md.. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known nucleotide sequence with other polynucleotide sequences from a variety of databases and "blastp" used to align a known amino acid sequence with one or more sequences from one or more databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences.

As used herein the term "NCBI" shall be taken to mean the database of the National Center for Biotechnology Information at the National Library of Medicine at the National Institutes of Health of the Government of the United States of America, Bethesda, MD, 20894.

In determining whether or not two nucleotide sequences fall within a particular percentage identity limitation recited herein, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art.

For example, nucleotide sequences may be aligned and their identity calculated using the BESTFIT program or other appropriate program of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux et al, Nucl. Acids Res. 12, 387-395, 1984). As discussed *supra* BLAST is also useful for aligning nucleotide sequences and determining percentage identity.

In another example of the invention, the membrane transport protein is a cystic fibrosis transmembrane regulator (CFTR) protein. As used herein the term "cystic fibrosis transmembrane regulator protein" or "CFTR" shall be taken to mean a protein that comprises an amino acid sequence at least about 80% identical to the sequence set forth in SEQ ID NO: 36. For example, the protein comprises an amino acid sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the amino acid sequence set forth in SEQ ID NO: 36.

In one example, the CFTR protein is a human CFTR protein.

Alternatively, or in addition, a CFTR protein is a protein encoded by a nucleic acid that comprises a nucleotide sequence at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 35. For example, the nucleic acid comprises a nucleotide sequence at least about 85% or at least about 90% or at least about 95% or at least about 98% or at least about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 35.

In one form of the invention, the CFTR protein is a mutant CFTR protein. For example, a CFTR mutation selected from the group consisting of 1717-1G→A, G542X, W1282X, N1303K, ΔF508, 3849+10kb C→T, 621+1 G→T, R553X, G551D, R117H, R1162X and R334W. For example, a CFTR protein comprising a ΔF508 mutation comprises an amino acid sequence set forth in SEQ ID NO: 61.

In another example of the invention the membrane transport protein is a mutant membrane transport protein. As used herein, the term "mutant membrane transport protein" shall be taken to mean a membrane transport protein that comprises one or more amino acid substitutions, insertions or deletions compared to a wild-type form of a membrane transport protein, e.g. a form of a membrane transport protein described *supra*. While it is not a requirement that the mutant membrane transport is functional,

it is beneficial that the membrane transport protein is capable of translocating to a plasma membrane to some degree.

For example, a mutant membrane transport protein has a reduced rate of transporter internalization. As used herein, the term "reduced rate of transporter internalization" shall be taken to mean that has been mutated in such a way that following translocation to the membrane it is not internalized or endocytosed, i.e. translocated away from the membrane at the same rate as the wild-type form of the membrane transport protein, rather it is internalized at a slower rate. For example, a mutant form of GLUT4 that has a reduced rate of transporter internalization includes the L489, 490A mutant (SEQ ID NO: 7) or the F5A mutant (SEQ ID NO: 9). Such a mutant is of use in the process of the present invention as it accumulates at the plasma membrane, effectively amplifying or increasing the level of membrane transport protein detected. Accordingly, such a mutant is useful for detection of a minor change (i.e. increase or decrease) of the translocation of a membrane transport protein, for example, when screening for a modulator of translocation of a membrane transport protein.

In the case of GLUT4, wild-type GLUT4 is more effectively translocated and recycled in the presence of insulin, as would be expected. Accordingly, wild-type GLUT4 is more effective in an assay for determining changes in translocation in the presence and/or absence of insulin, for example, when screening for a compound/agent that modulates GLUT4 translocation in the presence of insulin.

In one example of the invention, the membrane transport protein is a membrane transport protein that is rapidly translocated and recycled, whether that membrane transport protein is a wild-type or mutant form.

Detectable labels

In an example of the invention, the membrane transport protein is labeled. For example, with a detectable label. Accordingly, the present invention provides a process for determining the level of a labeled membrane transport protein translocated to the plasma membrane of a cell expressing the labeled membrane transport protein, said process comprising:

(a) determining the level of the labeled membrane transport protein at the plasma membrane of a cell using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind the label; and

(ii) determining the level of ligand bound to the labeled membrane transport protein;

(b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the labeled membrane transport protein within the cell with the ligand of the label for a time and under conditions sufficient for the ligand to bind the label; and

(ii) determining the level of ligand bound to the labeled membrane transport protein within the cell; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (ii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the level of the labeled membrane transport protein inside the cell.

For example, the label is a peptide, polypeptide or protein that is heterologous to the membrane transport protein. Such a label facilitates detection of the membrane transport protein with which the peptide, polypeptide or protein is associated.

A suitable detectable label includes, for example, a peptide, polypeptide or protein to which an antibody or ligand is capable of specifically binding. Alternatively, or in addition, the label is, for example, an enzyme that catalyzes a detectable reaction when contacted with a suitable substrate.

An example of a suitable detectable peptide polypeptide or protein is selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), an epitope tag described by Sloostra *et al., Mol. Drivers 2*: 156 - 164 (SEQ ID NO: 20 or SEQ ID NO: 21), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β-galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-shifted green fluorescent protein (SEQ ID NO: 29) and cyan fluorescent protein (SEQ ID NO: 30).

Alternatively, the membrane transport protein is labeled with a protein that directly associates with another known protein, such as for example, biotin, strepavidin or the

Strep-Tag, an 8 amino acid strepavidin binding sequence (WSHPQFEK, SEQ ID NO: 31) (available from Sigma-Genosys, Sydney, Australia).

In an exemplified embodiment of the invention, the label that is linked to a membrane transport protein is a HA tag (SEQ ID NO: 15).

In one form of the invention, the label is linked or fused to an extracellular domain of a membrane transport protein. Accordingly, it is preferable that the labeled membrane transport protein is a fusion protein. As used herein, the term "extracellular domain" shall be taken to mean the region or component of a protein that is located external to the cell when the membrane transport protein is incorporated in to the plasma membrane. Accordingly, when a membrane transport protein is not incorporated into the plasma membrane of a cell, the extracellular domain may be located within the cell.

Methods for determining the subcellular localization of a domain of a protein are known in the art. For example the following programs are useful for determining an extracellular domain of a protein:

i) PSORT, based on Horton and Nakai *Proc Int Conf Intell Syst Mol Biol.;5*:147-52, 1997) is available from the Brinkman Laboratory at Simon Fraser University, Burnaby, British Columbia, Canada;

ii) TopPred 2 based on Gunnar von Heijne, *J. Mol. Biol. 225*, 487-494, 1992 available from Stockholm University;

iii) HMMTOP based on Tusnády and Simon *J. Mol. Biol. 283:* 489-506, 1998 available from The Institute of Enzymology, Hungarian Academy of Sciences, Budapest; and

iv) SOSUI available from Department of Biotechnology, Tokyo University of Agriculture and Technology.

Alternatively, or in addition, a region of a membrane transport protein that is extracellular is predicted using the method described, for example, in Nakashima and Nishikawa, *FEBS Lett. 303*: 141-146, 1992; Nakashima and Nishikawa, *J. Mol. Biol., 238*: 54-61, 1994; Rost *et al, Prot Sci., 4*: 521-533, 1995; or Chou and Cai, *Biochem Biophys Res Commun. 320*:1236-9, 2004. Such methods rely upon the analysis of the amino acid composition of a membrane transport protein to determine, for example, hydropathy of regions of the protein to determine a region that is extracellular or intracellular.

In an exemplified form of the invention, the tag is linked or fused to the first exofacial or extracellular loop of the GLUT4 protein or a mutant thereof. For example, This protein comprises the sequence set forth in SEQ ID NO: 4 and/or is encoded by a nucleic acid set forth in SEQ ID NO: 3. A labeled TAIL mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 6. A labeled L489, 490A mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 8. A labeled F5A mutant of GLUT4 comprises, for example, the sequence set forth in SEQ ID NO: 10.

In an example of the invention, the label is covalently linked to the membrane transport protein. For example, a disulfide bond is formed between the label and the membrane transport protein. As will be apparent to the person skilled in the art such a membrane transport protein is then be delivered to the cell. In one embodiment the peptide encoded by the nucleic acid fragment of the present invention is expressed as a fusion protein with a peptide sequence capable of enhancing, increasing or assisting penetration or uptake of the protein by cells. Means and methods of enhancing, increasing or assisting penetration or uptake of the membrane transport protein by cells are described, for example, In Morris *et al, Nature Biotechnology 19,* 1173-1176, 2001.

In an alternative example, the membrane transport protein is expressed as a fusion protein with the label (e.g., as a recombinant fusion protein). As will be apparent to the skilled artisan, a fusion protein is advantageously expressed within a cell using an expression construct. As used herein, the term "expression construct" is to be taken in its broadest context and includes a promoter sequence that is placed in operable connection with a nucleic acid that encodes a membrane transport protein (e.g., a labeled membrane transport protein) of the present invention.

The term "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (i.e. upstream activating sequences, transcription factor binding sites, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue specific manner. In the present context, the term "promoter" is also used to describe a recombinant, synthetic or fusion molecule, or derivative which confers, activates or enhances the expression of a nucleic

acid molecule to which it is operably linked, and which encodes the peptide or protein. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or temporal expression of said nucleic acid molecule.

Placing a nucleic acid molecule under the regulatory control of, i.e., "in operable connection with", a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the coding sequence that they control. To construct heterologous promoter/structural gene combinations, it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the gene from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Typical promoters suitable for expression in a virus of a mammalian cell, or in a mammalian cell, mammalian tissue or intact mammal include, for example a promoter selected from the group consisting of, a retroviral LTR element, a SV40 early promoter, a SV40 late promoter, a cytomegalovirus (CMV) promoter, a CMV IE (cytomegalovirus immediate early) promoter, an $EF_{1\alpha}$ promoter (from human elongation factor 1α), an EM7 promoter or an UbC promoter (from human ubiquitin C).

Typical promoters suitable for expression in viruses of bacterial cells and bacterial cells such as for example a bacterial cell selected from the group comprising *E. coli, Staphylococcus sp, Corynebacterium sp., Salmonella sp., Bacillus sp.,* and *Pseudomonas sp.*, include, but are not limited to, the *lacz* promoter, the Ipp promoter, temperature-sensitive λ_L or λ_R promoters, T7 promoter, T3 promoter, SP6 promoter or semi-artificial promoters such as the IPTG-inducible *tac* promoter or lacUV5 promoter. A number of other gene construct systems for expressing the nucleic acid fragment of the invention in bacterial cells are well-known in the art and are described for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN

047 150338, 1987) and (Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001).

Typical promoters suitable for expression in yeast cells such as for example a yeast cell selected from the group comprising *Pichia pastoris, S. cerevisiae* and *S. pombe*, include, but are not limited to, the *ADH1* promoter, the *GAL1* promoter, the *GAL4* promoter, the *CUP1* promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

Methods for producing expression constructs are known in the art and are described, for example, in Ausubel *et al* (*In*: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) or Sambrook *et al* (*In*: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001).

In one embodiment, the expression construct forms a component of an expression vector. The term "expression vector" refers to a nucleic acid molecule that has the ability to confer expression on a nucleic acid to which it is operably connected, in a cell or in a cell free expression system. Within the context of the present invention, it is to be understood that an expression vector may comprise a promoter as defined herein, a plasmid, bacteriophage, phagemid, cosmid, virus sub-genomic or genomic fragment, or other nucleic acid capable of maintaining and or replicating heterologous DNA in an expressible format. Many expression vectors are commercially available for expression in a variety of cells. Selection of appropriate vectors is within the knowledge of those having skill in the art.

For example, expression vectors that contain suitable promoter sequences for expression in mammalian cells or mammals include, but are not limited to, the pcDNA vector suite supplied by Invitrogen, the pCI vector suite (Promega), the pCMV vector suite (Clontech), the pM vector (Clontech), the pSI vector (Promega) or the VP16 vector (Clontech).

Numerous expression vectors for expression of recombinant polypeptides in bacterial cells and efficient ribosome binding sites have been described, such as for example, PKC30 (Shimatake and Rosenberg, *Nature 292*, 128, 1981); pKK173-3 (Amann and Brosius, *Gene 40*, 183, 1985), pET-3 (Studier and Moffat, *J. Mol. Biol. 189*, 113,

1986); the pCR vector suite (Invitrogen), pGEM-T Easy vectors (Promega), the pL expression vector suite (Invitrogen) the pBAD/TOPO (Invitrogen, Carlsbad, CA); the pFLEX series of expression vectors (Pfizer Inc., CT,USA); the pQE series of expression vectors (QIAGEN, CA, USA), or the pL series of expression vectors (Invitrogen), amongst others.

Expression vectors for expression in yeast cells are know in the art and include, but are not limited to, the pACT vector (Clontech), the pDBleu-X vector, the pPIC vector suite (Invitrogen), the pGAPZ vector suite (Invitrogen), the pHYB vector (Invitrogen), the pYD1 vector (Invitrogen), and the pNMT1, pNMT41, pNMT81 TOPO vectors (Invitrogen), the pPC86-Y vector (Invitrogen), the pRH series of vectors (Invitrogen), pYESTrp series of vectors (Invitrogen).

Following production of a suitable gene construct, said construct is introduced into the relevant cell. Methods of introducing the gene constructs into a cell or organism for expression are well known to those skilled in the art and are described for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987) and (Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001). The method chosen to introduce the gene construct in depends upon the cell type in which the gene construct is to be expressed. Means for introducing recombinant DNA into bacterial cells include, but are not limited to electroporation or chemical transformation into cells previously treated to allow for said transformation, PEG mediated transformation, microinjection, transfection mediated by DEAE-dextran, transfection mediated by calcium phosphate, transfection mediated by liposomes such as by using Lipofectamine (Invitrogen) and/or cellfectin (Invitrogen), transduction by Adenoviuses, Herpesviruses, Togaviruses or Retroviruses and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agacetus Inc., WI, USA).

As exemplified herein, the present inventors have used a retroviral system to transfect or transduce a cell with an expression construct encoding a membrane transport protein. Accordingly, a viral delivery system is contemplated by the present invention.

Conventional viral based systems for the delivery of a nucleic acid include, for example, retroviral, lentivirus, adenoviral, adeno-associated virus and herpes simplex

virus. Viral vectors are an efficient and versatile method of gene transfer in target cells and tissues. Integration in the host cell genome occurs with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted expression construct. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. A lentiviral vector is a retroviral vector that is capable of transducing or infecting a non-dividing cell and typically produces high viral titers. Selection of a retroviral gene transfer system depends on the target tissue.

A Retroviral vector comprises cis-acting long terminal repeats (LTRs) with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the membrane transport gene into the target cell to provide long term transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., *J. Virol. 66*:2731-2739 (1992); Johann et al., *J. Virol. 66*:1635-1640 (1992); Sommerfelt *et al., Virol. 176*:58-59 (1990); Wilson *et al., J. Virol. 63*:274-2378 (1989); Miller *et al., J. Virol. 65*:2220-2224 (1991); PCT/US94/05700; Miller and Rosman *BioTechniques 7*:980-990, 1989; Miller, A. D. *Human Gene Therapy 1:*5-14, 1990; Scarpa *et al) Virology 180*:849-852, 1991; Burns *et al. Proc. Natl. Acad. Sci. USA 90*:8033-8037, 1993.).

In applications where transient expression of the nucleic acid is preferred, adenoviral based systems are typically used. Adenoviral based vectors are capable of high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. (see, e.g., West *et al., Virology 160*:38-47 1987; U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, *Human Gene Therapy 5*:793-801 1994; Muzyczka. *Clin. Invest. 94*:1351 1994).

Various adeno-associated virus (AAV) vector systems have also been developed for nucleic acid delivery. AAV vectors can be readily constructed using techniques known

in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski *et al. Molec. Cell. Biol. 8*:3988-3996, 1988; Vincent *et al.* (1990) Vaccines 90 (Cold Spring Harbor Laboratory Press); Carter *Current Opinion in Biotechnology 3*:533-539, 1992; Muzyczka. *Current Topics in Microbiol. and Immunol. 158*:97-129, 1992; Kotin, *Human Gene Therapy 5*:793-801, 1994; Shelling and Smith *Gene Therapy 1*:165-169, 1994; and Zhou et al. *J. Exp. Med. 179*:1867-1875, 1994.

Additional viral vectors useful for delivering a nucleic acid encoding membrane transport protein by gene transfer include those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus or an alphavirus or a conjugate virus vector (e.g. that described in Fisher-Hoch *et al., Proc. Natl. Acad. Sci. USA 86*:317-321, 1989).

As will be apparent from the preceding description, the present invention also encompasses providing the cell that expresses a membrane protein. The term "providing the cell that expresses a membrane protein" shall be taken to include transforming, transfecting or transducing a cell with an expression construct that encodes the membrane transport protein. Optionally, the term "providing the cell that expresses a membrane protein" shall be taken to additionally mean preparing the expression construct that encodes the membrane transport protein.

Suitable cells

As membrane transfer proteins are found in the majority of species any cell that expresses a membrane transport protein in nature is suitable for the performance of the instant invention. For example, transporters, channels and primary active transporters are found in bacterium, yeast, plants and mammals, see, for example, Chung *et al., Journal of Bacteriology, 183:* 1012-1021, 2001. Furthermore, ABC transport proteins are found in bacterium, yeast and mammals.

In an example of the invention, the cell is a eukaryotic cell, for example, a mammalian cell.

As will be apparent to the skilled artisan, the process of the present invention is preferably performed *in vitro*. Accordingly, the invention is performed, for example, using a cell isolated from a subject or using a cell line.

In one example of the invention, the method is performed in a cell that is amenable to transformation, transfection or transduction. For example, the cell is a cell selected from the group consisting of COS, CHO, murine 10T, MEF, NIH3T3, MDA-MB-231, MDCK, HeLa, K562, HEK 293, 3T3-L1 and 293T.

COS cells have been previously shown to be amenable to both transfection/transduction and the study of translocation of a membrane transport protein, particularly a GLUT4 protein.

In another example, a cell useful for performance of the process of the invention is a cell that is known to express and/or translocate the membrane transport protein of interest in nature. For example, muscle cells and adipocyte cells are known to express and translocate GLUT4 in nature. Accordingly, a muscle cell selected from the group consisting of a C2C12 cell, a L8 cell, a L6 cell, a F3 cell, a 10T1/2 cell, a H9C2 cell and a BC3H cell is useful for the performance of the invention. Alternatively, or in addition, an adipocyte cell or a pre-adipocyte cell selected from the group consisting of a 3T3-L1 cell, a HIB1B cell and a PA26 cell is useful for the performance of the invention.

As GLUT1 is also expressed and translocated in a muscle cell the muscle cells described *supra* are useful for the performance of the process of the invention to assess the translocation of GLUT4.

The translocation of CFTR is, for example, studied in a cell line derived from a tissue affected in cystic fibrosis, e.g., a Calu-3 airway epithelium cell line or a T84 colonic cell line.

Alternatively, the translocation of a membrane transport protein is studied using a primary cell, i.e. a cell isolated from a subject. For example, methods of isolating an adipocyte, a pre-adipocyte, a fibroblast, a muscle cell or an airway epithelium cell are known in the art. For example, Katoh *et al., Folia Histochem Cytobiol. 32*:235-8, 1994 describe a method for isolating a pre-adipocyte cell from adipose tissue.

Detection of a membrane transport protein

To determine the level of a membrane transport protein at the plasma membrane of a cell, a ligand is selected that is capable of specifically binding the membrane transport, for example, a ligand capable of binding to the label of a labeled membrane transport protein.

As used herein the term "ligand" shall be taken in its broadest context to include any chemical compound, polynucleotide, peptide, protein, lipid, carbohydrate, small molecule, natural product, polymer, etc. that is capable of selectively binding, whether covalently or not, to one or more specific sites on a target molecule, e.g., a labeled membrane transport protein (e.g., a label associated with or bound to the membrane transport protein). The ligand may bind to its target via any means including hydrophobic interactions, hydrogen bonding, electrostatic interactions, van der Waals interactions, pi stacking, covalent bonding, or magnetic interactions amongst others.

In one example of the invention, the ligand is an antibody. As used herein the term "antibody" refers to intact monoclonal or polyclonal antibodies, immunoglobulin (IgA, IgD, IgG, IgM, IgE) fractions, humanized antibodies, or recombinant single chain antibodies, as well as fragments thereof, such as, for example Fab, F(ab)2, and Fv fragments.

Antibodies referred to herein are obtained from a commercial source, or alternatively, produced by conventional means. Commercial sources will be known to those skilled in the art. For example, Sigma-Aldrich (Sydney, Australia) sell monoclonal antibodies that specifically bind HA, FLAG, V5, polyhistidine, c-myc, GST, MBP, β-galactosidase, GFP or biotin. The present inventors have used an anti-HA monoclonal antibody to determine the level of translocation of a HA tagged membrane transport protein (eg., a HA-tagged GLUT4 protein).

High titer antibodies are preferred, as these are more useful commercially in kits for analytical, diagnostic and/or therapeutic applications. By "high titer" is meant a titer of at least about $1:10^3$ or $1:10^4$ or $1:10^5$. Methods of determining the titer of an antibody will be apparent to the skilled artisan. For example, the titer of an antibody in purified antiserum may be determined using an ELISA assay to determine the amount of IgG in a sample. Typically an anti-IgG antibody or Protein G is used in such an assay. The amount detected in a sample is compared to a control sample of a known amount of

purified and/or recombinant IgG. Alternatively, a kit for determining antibody may be used, e.g. the Easy TITER kit from Pierce (Rockford, IL, USA).

Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art, and are described, for example in, Harlow and Lane (*In:* Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988). In one such technique, an immunogen comprising the antigenic polypeptide is initially injected into any one of a wide variety of animals (e.g., mice, rats, rabbits, sheep, humans, dogs, pigs, chickens and goats). The immunogen is derived from a natural source, produced by recombinant expression means, or artificially generated, such as by chemical synthesis (e.g., BOC chemistry or FMOC chemistry).

A peptide, polypeptide or protein is optionally joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen and optionally a carrier for the protein is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and blood collected from said the animals periodically. Optionally the immunogen is injected in the presence of an adjuvant, such as, for example Freund's complete or incomplete adjuvant, lysolecithin and/or dinitrophenol to enhance the immune response to the immunogen. Monoclonal or polyclonal antibodies specific for the polypeptide are then be purified from the blood isolated from an animal by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (i.e., reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described *supra*. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngenic with the immunized animal. A variety of fusion techniques may be employed, for example, the spleen cells and myeloma cells may be combined with a nonionic detergent or electrofused and then grown in a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of

hybrids are observed. Single colonies are selected and growth media in which the cells have been grown is tested for the presence of binding activity against the polypeptide (immunogen). Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies are isolated from the supernatants of growing hybridoma colonies using methods such as, for example, affinity purification as described *supra*. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies are then harvested from the ascites fluid or the blood of such an animal subject. Contaminants are removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and/or extraction.

It is preferable that an immunogen used in the production of an antibody is one which is sufficiently antigenic to stimulate the production of antibodies that will bind to the immunogen and is preferably, a high titer antibody. For example, an immunogen may be an entire protein.

Alternatively, an immunogen consists of a peptide representing a fragment of a polypeptide. Preferably, an antibody raised to such an immunogen also recognizes the full-length protein from which the immunogen was derived, such as, for example, in its native state or having native conformation.

As discussed *supra* antibody fragments are contemplated by the present invention. The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments.

Papain digestion of an antibody produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment.

Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen binding fragments that are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As

used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and $F(ab')_2$ fragments.

An "Fv" fragment is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a non-covalent association (V_H -V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H -V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen.

A Fab fragment [also designated as F(ab)] also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

In another example, a ligand is a small molecule. Chemical small molecule libraries are available commercially or alternatively may be generated using methods known in the art, such as, for example, those described in U.S. Patent No. 5,463,564.

Alternatively, a ligand is a peptidyl ligand. A peptidyl ligand are conveniently made by standard peptide synthesis, such as the Merrifield method of synthesis (Merrifield, *J Am Chem Soc, 85*,:2149-2154, 1963) and the myriad of available improvements on that technology (see e.g., Synthetic Peptides: A User's Guide, Grant, ed. (1992) W.H.

Freeman & Co., New York, pp. 382; Jones (1994) The Chemical Synthesis of Peptides, Clarendon Press, Oxford, pp. 230.).

For example, a membrane transport protein is labeled with strepavidin and the peptidyl ligand is a peptide that comprises a strepavidin binding sequence, e.g. the amino acid sequence set forth in SEQ ID NO: 31.

Alternatively, the membrane transport protein is labeled with biotin and the ligand is strepavidin.

As will be apparent to the skilled artisan, a preferred ligand is not capable of independently entering a cell that has not been permeabilized or disrupted. Accordingly, when a cell with an intact plasma membrane is contacted with the ligand, said ligand will bind to the membrane transport protein in the plasma membrane, and not to the membrane protein within the cell to a significant degree.

However, the present inventors have shown that the ligand may be capable of entering the cell when bound to a membrane transport protein that recycles away from the membrane without significantly altering the efficacy of the test. In fact, such a ligand is useful for determining internalization and/or a rate of internalization of a membrane transport protein.

A ligand useful in the process of the present invention is, for example, labeled with a detectable marker. For example, a fluorescent label (e.g. FITC or Texas Red), a fluorescent semiconductor nanocrystal (as described in US 6,306,610), a radiolabel or an enzyme (e.g. horseradish peroxidase (HRP), alkaline phosphatase (AP) or β-galactosidase)

An example of a suitable fluorescent label include fluorescein (FITC), 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, 4'-6-diamidino-2-phenylinodole (DAPI), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7, fluorescein (5-carboxyfluorescein-N-hydroxysuccinimide ester), rhodamine (5,6-tetramethyl rhodamine). The absorption and emission maxima, respectively, for these fluors are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm).

In an exemplified form of the invention a suitable fluorescent label is, for example, a fluorescent label obtained from Molecular Probes, Eugene. OR, such as, for example Alexafluor®350, Alexafluor® 488, Alexafluor® 555, Alexafluor® 594 or Alexafluor® 647. Such an antibody may be purchased from a commercial source. Alternatively, Molecular Probes supplies kits for labeling an antibody or proteinaceous ligand with such a fluorescent label.

In another example, the label is a fluorescent nanocrystal. A fluorescent nanocrystal generally comprises a core composed of cadmium sulfide (CdS), cadmium selenide (CdSe), or cadmium telluride (CdTe). The size and shape of the core aids in determining the wavelength at which the nanocrystal fluoresce. Coating the core is a shell composed of a non-emissive transparent but structurally related material, for example, ZnS. Finally, such a fluorescent nanocrystal is coated to provide a carboxylate surface to which many biological and nonbiological moieties may be attached. Such a nanocrystal is then conjugated to a ligand of interest, eg., an antibody, for example using an antibody conjugation kit from Qdot® (Hayward, CA). By exciting the nanocrystal at the relevant wavelength, the crystal emits a fluorescent light that is detectable using a method known in the art and/or described herein.

In a further example, the label is an enzymatic label. For example, a ligand is conjugated to β-galactosidase. Following contacting the cell and/or membrane transport protein with such a ligand, the sample is contacted with, for example, 5-bromo-4-chloro-3-indol-beta-D-galaotopyranoside (x-gal). The resulting reaction causes a blue colored precipitate to form. Other enzymatic labels are know in the art and include, for example, alkaline phosphatase or horseradish peroxidase (HRP). Suitable substrates for such enzymes are known in the art and include, for example, hydrogen peroxide or 3-3,5,5'-tetramethylbenzidine (TMB).

In another example, the ligand that binds to the label is detected using another ligand, such as, for example, an antibody. For example the secondary antibody/ligand is capable of specifically binding to the ligand that binds to the label. The present inventors have used a mouse monoclonal antibody to bind a labeled membrane transport protein and an anti-mouse secondary antibody to detect binding of the mouse monoclonal antibody. Preferably, the secondary antibody is labeled with a detectable marker, such as, for example, a marker described *supra*.

Alternatively, a ligand that binds to a label or a secondary antibody/ligand is conjugated to, for example, biotin. Strepavidin is capable of binding to biotin with high affinity and specificity. Accordingly, strepavidin labeled with a detectable marker is useful for detecting the binding of the ligand that binds to a label or a secondary antibody/ligand. A suitable detectable marker will be apparent to the skilled artisan, for example, a marker described *supra*.

Detection methods

Methods for detecting the binding of the ligand to the label and/or the secondary antibody/ligand to the primary ligand are known in the art and/or described herein. For example, such detection methods are described in Scopes (*In:* Protein purification: principles and practice, Third Edition, Springer Verlag, 1994).

In one form of the invention, the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds the label for a time and under conditions sufficient for the antibody to bind and determining the level of bound antibody.

As will be apparent to the skilled artisan, the detection method used depends upon the type of label used.

For example, a standard solid-phase ELISA format is useful in determining the level of an enzyme labeled ligand or antibody.

In one form such an assay involves immobilizing or growing or incubating the cell *supra* onto a solid matrix, such as, for example a polystyrene or polycarbonate microwell or dipstick, a membrane, or a glass support (e.g. a glass slide). Preferably, the ELISA assay is performed upon the plate upon which the cells are grown.

An antibody or ligand that specifically binds the membrane transport protein or label is brought into direct contact with the cell, and forms a direct bond with any of the membrane transport protein or label present in said sample. This antibody is generally labeled with a detectable reporter molecule, such as for example, an enzyme (e.g. horseradish peroxidase (HRP)), alkaline phosphatase (AP) or β-galactosidase. Alternatively, a second labeled antibody can be used that binds to the first antibody.

Following washing to remove any unbound antibody the detectable marker is detected by the addition of a substrate, such as for example hydrogen peroxide, TMB, or toluidine, or 5-bromo-4-chloro-3-indol-beta-D-galaotopyranoside (x-gal).

The level of the membrane transport protein may be determined using a standard curve that has been produced using known quantities of the membrane transport protein (e.g. recombinant membrane transport protein).

In the case of a fluorescent label, a fluorescence linked immunosorbent assay (FLISA) is useful for determining the level of a labeled ligand or antibody in a sample. A FLISA is performed essentially as described *supra*_for the ELISA assay, however, a substrate is not required to detect the bound labeled ligand or antibody. Rather, following washing to remove any unbound ligand/antibody the sample is exposed to a light source of the appropriate wavelength and the level of fluorescence emitted by each sample determined. A FLISA is also known as an immunofluorescence assay (IFA). The present inventors have clearly exemplified this form of assay.

As will be apparent to the skilled artisan, other detection methods based on an immunosorbent assay are useful in the performance of the present invention. For example, an immunosorbent method based on the description *supra* using a radiolabel for detection, or a gold label (e.g. colloidal gold) for detection, or a liposome, for example, encapsulating NAD+ for detection (e.g., as described in Kumada *et al., Journal of Chemical Engineering of Japan, 34*: 943-947, 2001) or an acridinium linked immunosorbent assay.

In another example, the level of the labeled ligand or antibody is determined using immunohistochemistry and/or immunofluorescence. For example, a cell or tissue section that is to be analyzed is optionally fixed to stabilize and protect both the cell and the proteins contained within the cell. Preferably, the method of fixation does not disrupt or destroy the antigenicity of the membrane transport protein, thus rendering it undetectable. Methods for fixing a cell are known in the art and include for example, treatment with paraformaldehyde, treatment with alcohol, treatment with acetone, treatment with methanol, treatment with Bouin's fixative and treatment with glutaraldehyde. Following fixation a cell is incubated with a ligand or antibody capable of binding the membrane transport protein. As discussed *supra* the ligand or antibody may be labeled with a detectable marker. Alternatively, a second labeled

antibody that binds to the first antibody can be used to detect the first antibody. Following washing to remove any unbound antibody, the level of ligand or antibody bound to the membrane transport protein is determined using an appropriate means. Means for detecting a label vary depending upon the type of label used and will be apparent to the skilled artisan.

Methods using immunofluorescence are preferable, as they are quantitative or at least semi-quantitative. Methods of quantitating the degree of fluorescence of a stained cell are known in the art and described, for example, in Immunohistochemistry (Cuello, 1984 John Wiley and Sons, ASIN 0471900524).

A high-throughput method of immunohistochemical/immunofluorescent analysis of a biological sample are preferred. For example, the EIDAQ 100 - HTM system of Q3DM (San Diego, CA, USA) allows the rapid automatic analysis of a biological sample to determine the presence and/or level of a polypeptide of interest.

Determining the level of a membrane transport protein within a sample

Following determining the level of membrane transport protein that has translocated to the plasma membrane of a cell, the total amount of that membrane transport protein in the cell is determined using a method known in the art and/or described herein.

Accordingly, comparison of the level of the membrane transport protein that has translocated to the plasma membrane to the level of the membrane transport protein detected in the cell provides a relative estimate of the level of the membrane transport protein that has translocated to the plasma membrane as a function of total membrane transport protein (for example as a percentage of total membrane transport protein). Such an estimate effectively "normalizes" the results of such an assay, reducing inter-assay variability and allowing comparisons between multiple assays.

To determine the total amount of membrane transport protein in a cell, the plasma membrane is permeabilized or disrupted to allow the detection means, e.g. a ligand or antibody, to enter the cell and bind the membrane transport protein. In permeabilizing or disrupting a cell membrane it is important that the membrane transport protein within the cell is not significantly degraded.

Methods for permeabilizing a cell are known in the art and/or described herein.

For example, a cell or plasma membrane is contacted with an agent or compound that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur.

A suitable agent or compound that permeabilizes or disrupts a plasma membrane will be apparent to the skilled artisan. For example, a suitable agent or compound that permeabilizes or disrupts a plasma membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β-D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α-hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof.

Agents useful for disrupting or permeabilizing a membrane are commercially available from, for example, Sigma-Aldrich, Sydney, Australia. For example, saponin, n-octyl-glucopyranoside, n-Dodecyl β-D-maltoside, hexadecyltrimethylammonium bromide, streptolysin-O , α-hemolysin or tetanolysin are commercially available from Sigma Aldrich.

The present inventors contacted a cell with a suitable amount of saponin for a time and under conditions suitable to disrupt or permeabilize a plasma membrane. This method permeabilized the plasma membrane sufficiently to facilitate detection of the level of membrane transport protein within the cell.

Methods for using other agents for permeabilizing a plasma membrane will be apparent to the skilled artisan. For example, Palmer *et al., EMBO J. 17:* 1598-1605, 1998 describe the use of Streptolysin-O to disrupt or permeabilize the membrane of a cell. Gariglio *FEBS Lett. 44*, 330, 1974, described the use of N-Dodecanoyl-N-methylglycine sodium salt for the lysis of eukaryotic cells.

In an example of the invention a cell is fixed. Methods for fixing a cell are known in the art and/or described herein. In one example, the cell is fixed using a process comprising contacting a cell with a fixative for a time and under conditions suitable for cell fixation to occur.

Fixing a cell ensures that the contents of the cell are less likely to be degraded and/or maintain their native conformation thereby facilitating detection.

A suitable compound for fixing a cell will be apparent to the skilled artisan and includes, for example, a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol, glutaraldehyde, Bouin's fixative and mixtures thereof.

In one example of the invention, a cell is fixed at substantially the same time as the cell is permeabilized or disrupted. In another example, the cell is fixed prior to or after the cell is permeabilized or disrupted. In a further example, the cell is fixed in the absence of permeabilization or disruption.

Following permeabilization and/or fixation the level of a membrane transport protein is determined using a method known in the art and/or described *supra*.

Following determining the level of a membrane transport protein in a cell that comprises a membrane that has been permeabilized or disrupted, the level of the membrane protein at the surface of the protein relative to the level of membrane protein in a cell is determined. Accordingly, such a process enables a quantitative measurement of the level of a membrane transport protein that has translocated to the plasma membrane of a cell.

By determining the level of a membrane transport protein at the plasma membrane of a cell relative to or as a function of the level of the membrane transport protein in the cell, the process of the invention effectively standardizes or normalizes the detected levels of protein. The assay normalizes the level of translocated membrane transport protein based on the level of membrane transport protein in the assay. Such normalization facilitates comparison of results attained in separate/distinct assays.

Should the assay be performed using a plurality of cells, the assay may additionally be normalized, for example, for cell number. Such normalization accounts for variation in the number of cells in an assay (a variable that may affect the level of membrane protein detected in the assay).

Methods for determining cell number are known in the art, and include, for example, manually counting the number of cells used in an assay, or, alternatively, counting a fraction of the number of cells used in an assay. For example, when using a microtitre plate, the number of cells in a fraction of the total area of the plate (eg. 10% or 25% or 50%) of each well of the plate is counted, and this result used to estimate the number of cells in each well of the plate.

Alternatively, or in addition, a sample is normalized for cell number by detecting a protein that is expressed by the cells used in the assay. A protein useful in such an assay is one that is not affected by any conditions, eg., compounds, to which the cells are exposed. For example, should the cells be exposed to various concentrations of a compound, a protein that is affected by the compound (i.e., the expression levels of the protein) is not useful for normalization. Various proteins useful for normalization are known in the art and include, for example, β-tubulin, actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β2 microglobulin, hydroxy-methylbilane synthase, hypoxanthine phosphoribosyl-transferase 1 (HPRT), ribosomal protein L13c, succinate dehydrogenase complex subunit A and TATA box binding protein (TBP).

Methods for determining the level of a protein are described *supra* and are to be taken to apply *mutatis mutandis* to the detection of a control protein for normalization. For example, the level of a control protein for normalization is determined using an antibody based assay.

In one example of the invention, the number of cells in a sample is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a component of the cell for a time and under conditions to occur and determining the level of antibody or ligand bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of cell number.

Antibodies capable of binding to such control proteins are known in the art. For example, an anti-β-tubulin monoclonal antibody is available from Sigma-Aldrich (Sydney, Australia), as is an anti-actin polyclonal antibody or an anti-β2 microglobulin monoclonal antibody.

As the control proteins for normalization described *supra* are intracellular, such normalization is, for example, performed following disruption or permeabilization of the plasma membrane.

Alternatively, or in addition, the sample is normalized for cell number using a compound capable of passing across a cell membrane. For example, a DNA binding molecule, such as, for example Hoechst 33342, is capable of staining DNA in a cell with an intact plasma membrane. Clearly such a nucleic acid stain is also useful for normalization of a cell with a disrupted or permeabilized membrane. Alternative nucleic acid stains include, for example, propidium-iodide, 4' 6-diamidino-2-phenylindole (DAPI), Mithramycin, 7-Aminoactinomycin D or To-Pro-3.

The present inventors have shown that wheat germ agglutinin (WGA) is also useful for normalization for cell number. WGA is capable of binding N-acetylglucosamine or chitobiose. Both of these sugar structures are common to plasma membranes of many cells. Accordingly, WGA is useful for determining cell number or normalizing for cell number using either an undisrupted/unpermeabilized cell or a disrupted/permeabilized cell.

As will be apparent to the skilled artisan, the method need not determine or estimate the number of cells in a sample. Rather the method, for example, comprises determining the level of a ligand, antibody or compound used for detecting/estimating/normalizing for cell number in a sample and comparing this level to the level detected in another sample.

Accordingly, a method for normalizing for cell number comprises:

(i) contacting a sample comprising a plurality of cells of the invention with a ligand or antibody capable of binding to a cell or a component thereof for a time and under conditions sufficient for a complex to form between the cell or component thereof and the antibody or ligand and determining the level of the complex; and

(ii) contacting another sample comprising a plurality of cells of the invention with a ligand or antibody capable of binding to a cell or a component thereof for a time and under conditions sufficient for a complex to form between the cell or component thereof and the antibody or ligand and determining the level of the complex, wherein a level of the complex that is similar or comparable in (i) and (ii) indicates that there is a similar or comparable number of cells in the samples.

For example, the level of the complex that is similar or comparable in (i) and (ii) does not vary significantly.

As will be apparent to the skilled artisan the level of the complex detected may also be used to normalize the level of translocated membrane transport protein detected. For example, the level of the translocated membrane transport protein detected is expressed as a function of the level of the complex detected thereby normalizing for approximate cell number.

Induction of translocation

In an example of the invention, the process additionally comprises inducing translocation of the membrane transport protein. For example, the membrane transport protein is induced to translocate using a method comprising contacting a cell with an amount of peptide, polypeptide or protein sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur thereby inducing translocation of the membrane transport protein.

For example, contacting a cell with lactose or sucrose induces translocation of a lactose permease to a plasma membrane. Contacting a cell with a sufficient amount of isoproterenol induces translocation of the SCN5A sodium channel to the plasma membrane. Furthermore, contacting a cell with a secretagogue (e.g., KCl, ionomycin or a phorbol ester) induces translocation of a N-type Ca2+ channel to the plasma membrane of a cell.

Furthermore, the present inventors have shown that contacting a cell expressing a GLUT protein (e.g. a GLUT4 protein) with insulin induces increased translocation of the GLUT protein to the plasma membrane.

The present inventors have additionally demonstrated that by contacting a cell expressing a GLUT protein with an amount of insulin and sucrose to induce translocation enhanced levels of the GLUT protein are translocated to the plasma membrane. For example, levels of the GLUT protein translocated to the plasma membrane of a cell contacted with both sucrose and insulin are enhanced compared to the levels induced in a cell contacted with insulin alone.

Accordingly, the invention provides for induction of translocation of a GLUT protein or a mutant thereof by contacting a cell expressing said GLUT protein or mutant with an amount of insulin sufficient to induce translocation for a time and under conditions sufficient for translocation to occur.

In an example, the cell are additionally contacted with an amount of sucrose sufficient to induce translocation for a time and under conditions sufficient for translocation to occur.

In an example of the invention, a cell is contacted with sucrose and/or insulin in the presence of serum.

In one form of the invention, the cells are contacted with insulin and then contacted with sucrose. For example, the cells are contacted with between about 100nM insulin and about 700nM insulin, or between about 200nM insulin and about 600nM insulin, or about 200nM insulin, or about 400nM insulin or about 600nM insulin.

Cells with an enhanced level of the membrane transport protein translocated to the plasma membrane are useful for, for example, screening for modulators of translocation of the membrane transport protein. Clearly, such an assay is more sensitive than an assay that does not enhance the level of membrane transport protein at the cell surface. This is because the level of the plasma membrane transport protein at the cell surface is enhanced, thereby facilitating detection.

Furthermore, such an assay is useful for selecting for a potent inhibitor of translocation of a membrane transport protein.

Furthermore, the present inventors have clearly demonstrated that the process of the invention is useful for screening for modulators of the level of translocation of a plasma membrane protein. In particular, the present inventors have demonstrated that contacting a cell with insulin or contacting a cell with insulin and then sucrose are useful for enhancing the level of a GLUT4 protein translocated to the plasma membrane of a cell.

Alternative methods for the induction of translocation of GLUT4 to the plasma membrane include, for example, contacting a cell with a sufficient amount of

margatoxin or another voltage-gated K+ channel, Kv1.3 antagonist for a time and under conditions sufficient to suppress expression or activity of voltage-gated K+ channel, Kv1.3. Such suppression of activity (using margatoxin) or expression (using a mouse knock-out) has been shown to increase the level of GLUT4 translocated to the plasma membrane of a cell (Xu et al, Proc *Natl Acad Sci U S A. 101*:3112-3117, 2004.)

Suppression of translocation

The present inventors have additionally suppressed the level of a membrane transport protein translocated to the plasma membrane of a cell. Such a method is useful for, for example, modeling a disease/disorder or condition that is associated with a reduced or suppressed level of translocation of a plasma membrane protein. This model is then useful for determining a modulator or putative therapeutic of such a disease/disorder or condition.

For example, the present inventors have shown that by incubating cells expressing GLUT4 in the absence of insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation the level of GLUT4 translocated to the plasma membrane of the cell in the presence of insulin is suppressed. For example, a cell is incubated in the presence of insulin for at least about 16 hours to at least about 72 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for at least about 24 hours to at least about 48 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for about 24 hours prior to induction of translocation or testing of a compound/agent. For example, a cell is incubated in the presence of insulin for about 48 hours prior to induction of translocation or testing of a compound/agent.

Conditions sufficient to induce resistance to insulin include, for example, the absence of insulin. Accordingly, an example of the invention provides for contacting a cell with insulin in the absence of serum for a time and under conditions to induce resistance to GLUT4 translocation. A cell that is resistant to insulin induced GLUT4 translocation is useful as a model for determining or identifying or isolating a modulator of insulin resistance, such as, for example, non-insulin dependent diabetes mellitus (NIDDM, type II diabetes).

Other methods for inducing resistance to translocation of a membrane transport protein will be apparent to those skilled in the art. For example, resistance to insulin induced translocation of a GLUT protein other than GLUT4 or a mutant thereof is induced using a method essentially as described *supra*.

Parallel cellular samples

One form of the present invention provides for performing the present invention in parallel cellular samples. Accordingly, the present invention provides a process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said process comprising:

(a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:

(i) contacting a cell with a ligand that binds to the extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind the labeled membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) determining the level of membrane transport protein in another cell using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the membrane transport protein with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;

(iii) determining the level of ligand bound to the membrane transport protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the membrane transport protein at the plasma membrane relative to the total level of membrane transport protein.

As described *supra*, an example of the invention utilizes a labeled membrane transport protein to facilitate detection of the protein. Accordingly, the present invention provides a process for determining the level of a labeled membrane transport protein translocated to the plasma membrane of a cell, said process comprising:

(a) determining the level of the labeled membrane transport protein at the plasma membrane of a cell using a method comprising:

(i) contacting a cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind the labeled membrane transport protein; and

(ii) determining the level of ligand bound to the labeled membrane transport protein;

(b) determining the level of labeled membrane transport protein in another cell using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled membrane transport protein with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind the labeled membrane transport protein;

(iii) determining the level of ligand bound to the labeled membrane transport protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.

As used herein, the term "parallel cellular sample" shall be taken to mean that the cells used in the performance are grown under essentially or substantially the same conditions. Accordingly, cells are grown in, for example, the same or similar growth medium and/or grown at approximately the same temperature and/or grown in the same concentration of CO_2. Preferably, the cells are also isogenic.

As used herein, the term "isogenic" shall be taken to refer to cells that are derived from a clonal cell line. Accordingly, such cells are substantially identical at the genetic level. Preferably, each of the cells is from the same cell line.

For example, a cell that expresses a recombinant membrane transport protein preferably comprises an expression construct (encoding the recombinant membrane transport protein) that has stably integrated into the genome of the cell. Such stable integration means that cells derived from the original cell also comprise the expression construct and express the encoded protein. Furthermore, stable integration of the expression construct facilitates a standard or relatively unvarying level of expression of the membrane transport protein in cells derived from the original cell.

By culturing cells in parallel comparisons are made more reproducible. This is because variables controlled or influenced by the environment in which a cell is grown or cultured, such as, for example, gene expression levels are essentially controlled. Accordingly, a direct comparison between the level of a membrane transport protein at the cell surface of one cell compared to the level of a membrane transport protein in another (isogenic) cell cultured under essentially the same conditions facilitates determining the level of the membrane transport protein translocated to the plasma membrane as a function of the level of the membrane transport protein in the cell.

Methods for determining the level of a ligand bound to a membrane transport protein and/or the level of a membrane transport protein are described *supra* and are to be taken to apply *mutatis mutandis* to the method for determining the level of a membrane transport protein translocated to the plasma membrane of a cell using a plurality of cells.

In one example, the process of the invention is performed in a plurality of cells. In accordance with this example, the inventive assay additionally comprises normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of the ligand bound to the membrane transport protein is determined. Methods for normalizing the determined level of ligand bound to the membrane transport protein are described *supra*.

Such normalization facilitates not only inter assay comparisons but also for determining the level of translocation of a membrane transport protein using cells cultured in, for example, parallel.

In an exemplified form of the invention, the inventors contacted a sample comprising cells with a labeled wheat germ agglutinin (WGA) for a time and under conditions sufficient for the WGA to bind to its ligand in the plasma membrane of a cell, and determining the level of WGA in the sample. For example, the sample is washed to remove any unbound WGA prior to detection. The level of WGA detected in the sample facilitates normalization of the level of the level of membrane transport protein detected relative to cell number. Clearly this facilitates determining the level of translocation of a membrane transport protein in addition to facilitating comparison between different samples.

Using the method of the present invention, the present inventors have produced a method for determining the level of a labeled GLUT4 protein or mutant thereof translocated to the plasma membrane of a cell. Accordingly, the present invention provides a process for determining the level of a labeled GLUT4 protein or labeled mutant GLUT4 protein translocated to the plasma membrane of a cell, said process comprising:

(a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(b) determining the level of membrane transport protein in another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

Furthermore, the present inventors have adapted this method to determine the level of a labeled GLUT4 protein or mutant thereof translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation. Accordingly, the present invention additionally provides a process for determining the level of the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, said process comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;

(b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell (a) using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(c) determining the level of membrane transport protein in another cell (a) using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) detecting the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

Methods for inducing resistance to GLUT4 translocation are described *supra* and are to be taken to apply *mutatis mutandis* to the instant example of the method of the invention.

As will be apparent to the skilled artisan the use of a labeled membrane transport protein is a model for the translocation of a wild-type or unlabeled membrane transport protein. For example, the label does not affect the function and/or translocation of the labeled membrane transport protein.

Determining recycling of a membrane transport protein

As a membrane transport protein is also recycled or turned-over from the plasma membrane of a cell (i.e. the membrane transport protein is removed from the

membrane) the present invention additionally provides a method for determining the level or rate of recycling of a membrane transport protein in a cell. Accordingly, the present invention additionally provides A process for determining the level of recycling of a membrane transport in a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to determine the level of recycling of the membrane transport protein in the cell.

In another example, the present invention provides a process for determining a change in the level of recycling of a membrane transport in a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b),

wherein a change in the level of the membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

As will be apparent to the skilled artisan an increase in the level of the membrane transport protein translocated to the plasma membrane at (b) compared to (a) is indicative of an enhanced level of recycling of the membrane transport protein. In contrast, a reduction in the level of the membrane transport protein at (b) compared to (a) is indicative of an enhanced level of recycling of the membrane transport protein.

By determining the change in the level of the membrane transport protein at the plasma membrane at (a) and (b) and optionally expressing this as a function the rate of recycling of the membrane transport protein is determined. Clearly the present invention extends to determining the level of recycling of the membrane transport

protein at a number of points in time and determining the rate of recycling of the membrane transport protein.

In one form of the invention, the cells are contacted with the ligand of the label throughout the process. The present inventors have shown that following binding of the ligand to the label, recycling of the membrane transport protein is not altered.

The methods described *supra* are also useful for determining the rate and/or level of internalization of a membrane transport protein. For example, a cell is incubated in the presence of an agent that induces translocation of the membrane transport protein to the plasma membrane and then the agent is removed. By determining the level of the membrane transport protein at the plasma membrane at a plurality of points of time following the removal of the agent the level and/or rate of internalization of the membrane transport protein is determined.

Accordingly, the present invention provides a method for determining the level of internalization of a membrane transport protein comprising:

(a) inducing translocation of a membrane transport protein by a method comprising contacting a plurality of cells with one or more peptides, polypeptides, proteins or compounds that induces translocation of the membrane transport protein for a time and under conditions for translocation to occur;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell (a) using the process of the invention;

(c) determining the level of the membrane transport protein translocated to the plasma membrane of another cell (a) using the process of the invention, wherein the other cell is cultured for a longer period of time than the cell (b); and

(d) comparing the level of the membrane transport protein translocated to the plasma membrane at (b) and (c),

wherein the level of the membrane transport protein translocated to the plasma membrane at (b) compared to (c) indicates the level of internalization of the membrane transport protein.

Clearly this method applies *mutatis mutandis* to a method for determining the rate of internalization of a membrane transport protein.

Mutations affecting translocation of a membrane transport protein

The process of the present invention is also useful for determining or identifying a mutation in a nucleic acid that encodes a membrane transport protein wherein the mutation affects the translocation of the membrane transport protein. Accordingly, the present invention provides a method for determining a mutation in a nucleic acid encoding a mutant membrane transport protein, wherein said mutation modulates translocation of said membrane transport protein, said method comprising:

(a) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process of the invention; and

(b) determining the level of a wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process of the invention,

wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that modulates the level of level of translocation of the membrane transport protein to the plasma membrane.

As will be apparent to the skilled artisan, this method may also be adapted to determine the level of recycling or internalization essentially as described *supra*.

In one form of the invention both the mutant and wild-type form of the membrane transport protein are expressed in the same cell. As will be apparent to the skilled artisan, labeling each of the membrane transport proteins with a different label facilitates detection of each protein.

In another form of the invention, the mutant and wild-type form of the membrane transport protein are expressed in different cells. Accordingly, each membrane transport protein may be with the same label.

In one form of the invention, the process additionally comprises providing a cell expressing a mutant membrane transport protein and/or a wild-type form of the membrane transport protein. Methods for providing a cell, e.g. production of an expression construct and/or transforming/transfecting the expression construct into a cell are known in the art and described, for example, *supra*.

A mutant or mutated form of a membrane transport protein is isolated from a subject suffering from, for example, a disorder thought to be associated with aberrant translocation of a membrane transport protein.

Alternatively, or in addition, a mutant form of a membrane transport protein is produced using recombinant means. Means for producing a mutation in a nucleic acid are known in the art and include for example, site-directed mutagenesis or PCR mediated mutagenesis. Such methods are described, for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987), Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001) or Dieffenbach (ed) and Dveksler (ed) (*In*: PCR Primer: A Laboratory Manual, Cold Spring Harbour Laboratories, NY, 1995).

The present inventors have produced various mutations in a cDNA encoding GLUT4 by, for example, site-directed mutagenesis or replacing regions of GLUT4 with regions from GLUT3. Furthermore, the present inventors have shown that these mutations affect the level of translocation of the mutant membrane transport protein.

In an example of the invention, the process additionally comprises determining the level of an expression product (e.g., mRNA or protein) encoded by the mutant and/or nucleic acid. Determining the level of expression of each nucleic acid facilitates comparing said expression levels to determine a compound that modulates the level of translocation of a membrane transport protein rather than modulating the level of expression of a membrane transport protein. Methods for determining expression levels are known in the art and/or are described, for example, in Ausubel *et al* (In: Current Protocols in Molecular Biology. Wiley Interscience, ISBN 047 150338, 1987), Sambrook *et al* (In: Molecular Cloning: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York, Third Edition 2001) or Scopes (*In:* Protein purification: principles and practice, Third Edition, Springer Verlag, 1994).

Modulatory agents

The present invention provides an assay that is easily amenable to a process for the identification of compounds that modulate the level of translocation of a membrane transport protein. For example, the present inventors have shown that the process of the invention may be performed in a 384 well format thereby facilitating high-throughput screening for a modulatory compound. Accordingly, the present invention additionally provides a process for determining an agent that modulates translocation of

a membrane transport protein to the plasma membrane of a cell, said process comprising:

(a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention; and

(b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention,

wherein a difference in the level of a membrane transport protein translocated to the plasma membrane of a cell at (b) compared to (a) indicates that the candidate agent modulates translocation of the membrane transport protein.

As will be apparent to the skilled artisan an agent that enhances the level of membrane transport protein at (b) compared to (a) enhances the level of translocation of the membrane transport protein. In contrast an agent that reduces the level of membrane transport protein at (b) compared to (a) reduces the level of translocation of the membrane transport protein

The agent may be derived from any source. For example, a test agent can be a pharmacologic agent already known in the art or can be an agent previously unknown to have any pharmacological activity. The agent can be naturally occurring or designed in the laboratory. The agent can be isolated from microorganisms, animals, or plants, or can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des. 12, 145:* 1997.

Methods for the synthesis of molecular libraries are known in the art (see, for example, DeWitt *et al., Proc. Natl. Acad. Sci. U.S.A. 90*: 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*: 11422, 1994; Zuckermann *et al., J. Med. Chem. 37*: 2678, 1994; Cho *et al., Science 261:* 1303, 1993; Carell *et al., Angew. Chem. Int. Ed. Engl. 33*: 2059, 1994;

Carell *et al., Angew. Chem. Int. Ed. Engl. 33*: 2061; Gallop *et al., J. Med. Chem. 37*: 1233, 1994). Libraries of compounds are, for example, presented in solution (see, e.g., Houghten, *Bio Techniques 13*: 412-421, 1992), or on beads (Lam, *Nature 354*: 82-84, 1991), chips (Fodor, *Nature 364*: 555-556, 1993), bacteria or spores (Ladner, U.S. Pat. No. 5,223,409), plasmids (Cull *et al., Proc. Natl. Acad. Sci. U.S.A. 89*: 1865-1869, 1992), or:phage (Scott & Smith, *Science 249*: 386-390, 1990; Devlin, *Science 249*: 404-406, 1990); Cwirla *et al., Proc. Natl. Acad. Sci. 97*: 6378-6382, 1990; Felici, J. *Mol. Biol. 222*: 301-310, 1991; and Ladner, U.S. Pat. No. 5,223,409).

Alternatively, an agent is isolated from a natural compound library. Such a natural compound library is commercially available from, for example, InterBioscreen, Moscow, Russia.

The present inventors have shown that the fungal metabolite wortmannin is capable of suppressing GLUT4 translocation to the plasma membrane of a cell.

In one form of the invention a candidate agent is, for example an antibody or fragment thereof. Such an antibody is preferably capable of binding to and inhibiting the activity of a gene that is associated with or controls translocation of a membrane transport protein to the plasma membrane of a cell.

For example, the membrane transport protein is GLUT4 and the antibody binds to voltage-gated K+ channel, Kv1.3 thereby inhibiting the activity of the channel. Inhibition of the activity of this ion channel has been previously shown to enhance GLUT4 translocation to the plasma membrane.

In another form of the invention, the agent is an antisense nucleic acid, and RNAi molecule, a shRNA molecule or a ribozyme.

The term "antisense nucleic acid" shall be taken to mean DNA or RNA molecule that is complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American 262*:40, 1990) and capable of interfering with a post-transcriptional event such as mRNA translation. The use of antisense methods is known in the art (Marcus-Sakura, *Anal. Biochem. 172*: 289, 1988). Preferred antisense nucleic acid will comprise a nucleotide sequence that is complementary to at least 15 contiguous nucleotides of a sequence encoding the amino acid of the protein of interest.

As used herein, the term "ribozyme" shall be taken to refer to a nucleic acid molecule having nuclease activity for a specific nucleic acid sequence. To achieve specificity, preferred ribozymes will comprise a nucleotide sequence that is complementary to at least about 12-15 contiguous nucleotides of a sequence encoding a protein that modulates the translocation of a membrane transport protein.

As used herein, the terms "small interfering RNA" ('siRNA"), short hairpin RNA ("shRNA"), and "RNAi" refer to homologous double stranded RNA (dsRNA) that specifically targets a gene product, thereby resulting in a null or hypomorphic phenotype. Specifically, the dsRNA comprises two short nucleotide sequences derived from the target RNA and having self-complementarity such that they can anneal, and interfere with expression of a target gene, presumably at the post-transcriptional level. RNAi molecules are described by Fire et al., Nature 391: 806-811, 1998, and reviewed by Sharp, Genes & Development, 13: 139-141, 1999). As will be known to those skilled in the art, short hairpin RNA ("shRNA") is similar to siRNA, however comprises a single strand of nucleic acid wherein the complementary sequences are separated an intervening hairpin loop such that, following introduction to a cell, it is processed by cleavage of the hairpin loop into siRNA. Accordingly, each and every embodiment described herein is equally applicable to siRNA and shRNA.

Preferred siRNA or shRNA molecules comprise a nucleotide sequence that is identical to about 19-21 contiguous nucleotides of the target mRNA. Preferably, the target sequence commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-60%, more preferably 40-60% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target sequence in the genome of the animal in which it is to be introduced, e.g., as determined by standard BLAST search.

Methods for determining the level of translocation of a membrane transport protein are described *supra* and are taken to apply *mutatis mutandis* to the present method of the invention.

In one example, the method of the invention additionally comprises determining whether or not the agent is toxic. In accordance with this embodiment, the cells are screened to determine viability. Methods for determining viability include, for

example, contacting a cell with a labeled agent that is incorporated or taken up by the cell for a time and under conditions sufficient for the cell to take up or incorporate the agent and detecting the label. Alternatively, the method comprises contacting a cell with a compound that is metabolized by the cell for a time and under conditions sufficient for the cell to metabolize the compound and detecting the metabolite.

For example, a cell viability assay comprises determining the level of ^{3}H thymidine by a cell. Alternatively, trypan blue staining is useful for determining cell viability. Alternatively, or in addition, colorimetric assays such as for example, the ProCheck™ assay is available from Serologicals. A variety of other cell viability assays are known in the art and described for example, in Animal Cell Culture: Practical Approach, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970.

For example, cell viability is measured using a methylthiazol tetrazolium (MTT) reduction assay (Mossman, *J. Immunol. Meth., 65*: 55, 1983). MTT is reduced by mitochondrial dehydrogenases in living cells; this reaction produces formazan crystals which are quantified by photometry after extraction. For example, using this method, an IC50 (concentration that reduces cell viability by 50 %) is calculated.

Neutral red staining is also useful for determining cell viability. Neutral red is accumulated in the lysosomes in living cells that become colored by the dye. The dye is extracted and quantified using densitometry.

Alternatively, or in addition, cell viability is determined by determining the level of lactate dehydrogenase activity (Legrand *et al., J. Biotechnol. 25*:231-43, 1992). Lactate Dehydrogenase is a cytosolic enzyme that is released upon cell lysis. For example, an IC50 (concentration that reduces cell viability by 50 %) can be calculated. This assay evidences chemicals inducing alterations in cell integrity (lysis). Kits for determining lactate dehydrogenase levels are commercially available from, for example, Promega or Vinci-Biochem, Vinci, Italy.

In one example, the present invention provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

(a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;

(b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of the invention, wherein a difference in the level of a membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.

(c) optionally, determining the structure of the candidate agent; and

(d) providing the candidate agent or the name or structure of the candidate agent.

Naturally, for agents that are known albeit not previously tested for their function using a screen provided by the present invention, determination of the structure of the compound is implicit in step (i) *supra*. This is because the skilled artisan will be aware of the name and/or structure of the compound at the time of performing the screen.

As used herein, the term "providing the agent" shall be taken to include any chemical or recombinant synthetic means for producing said agent or alternatively, the provision of an agent that has been previously synthesized by any person or means.

For example, a peptidyl compound is synthesized using is produced synthetically. Synthetic peptides are prepared using known techniques of solid phase, liquid phase, or peptide condensation, or any combination thereof, and can include natural and/or unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (Nα-amino protected Nα-t-butyloxycarbonyl) amino acid resin with the deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield, *J. Am. Chem. Soc., 85*:2149-2154, 1963, or the base-labile Nα-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids described by Carpino and Han, *J. Org. Chem., 37*:3403-3409, 1972. Both Fmoc and Boc Nα-amino protected amino acids can be obtained from various commercial sources, such as, for example, Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs.

Synthetic peptides are alternatively produced using techniques known in the art and described, for example, in Stewart and Young (*In:* Solid Phase Synthesis, Second

Edition, Pierce Chemical Co., Rockford, Ill. (1984) and/or Fields and Noble (*Int. J. Pept. Protein Res., 35*:161-214, 1990), or using automated synthesizers. Accordingly, peptides of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various unnatural amino acids (e.g., β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine.

In another embodiment, a peptidyl agent is produced using recombinant means. For example, an oligonucleotide or other nucleic acid (eg., a nucleic acid encoding a dominant negative inhibitor of the protein of interest) is placed in operable connection with a promoter. Methods for producing such expression constructs, introducing an expression construct into a cell and expressing and/or purifying the expressed peptide, polypeptide or protein are known in the art and described *supra*.

Alternatively, the peptide, polypeptide or protein is expressed using a cell free system, such as, for example, the TNT system available from Promega. Such an *in vitro* translation system is useful for screening a peptide library by, for example, ribosome display, covalent display or mRNA display.

Methods for producing antibodies, preferably a monoclonal antibody, or a fragment or recombinant fragment thereof are described *supra*.

In a preferred embodiment, the compound or modulator or the name or structure of the compound or modulator is provided with an indication as to its use e.g., as determined by a screen described herein.

In another example, the invention provides a process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

(a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process of the invention;

(b) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process of any one of the invention, wherein a difference in the level of a

membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(d) providing, the candidate agent.

In one example, the candidate agent is provided with an indication as to its use, for example, as determined using a method described herein.

The present inventors have additionally produced a method for modeling insulin resistance. For example, the present inventors have produced a model in which a cell is resistant to insulin induced GLUT4 translocation. Accordingly, the present invention additionally provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention; and

(c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention,

wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

Conditions associated with insulin resistance include, for example, Syndrome X, type II diabetes (non-insulin dependent diabetes mellitus (NIDDM), hypertension, cardiovascular disease or obesity. Accordingly, an agent identified or determined using the method of the present invention is, for example, useful for the treatment of such a condition.

In one example, the agent is provided with an indication as to its use, for example, as determined using a method described herein.

The present invention additionally provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;

(c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment of diabetes;

(d) optionally, determining the structure of the candidate agent; and

(e) providing the candidate agent or the name or structure of the candidate agent.

In one example, the agent is provided with an indication as to its use, for example, as determined using a method described herein.

Furthermore, the present invention provides a process for determining a candidate compound for the treatment of insulin resistance comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;

(c) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment of diabetes;

(d) optionally, determining the structure of the candidate agent;

(e) optionally, providing the name or structure of the candidate agent; and

(e) providing the candidate agent.

Suitable agents are known in the art and/or described *supra*.

Furthermore, methods for determining the level of translocation of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell are known in the art and/or described herein.

For example, the method of the invention is useful for determining an agent for the treatment of diabetes, e.g., NIDDM.

Accordingly, the present invention additionally provides a process for manufacturing a medicament for the treatment of insulin resistance comprising:

(a) determining a candidate compound for the treatment of insulin resistance using a process comprising:

(i) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with insulin for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell;

(ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell (a) in the absence of a candidate agent by performing the process of the invention;

(iii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell (a) in the presence of the candidate agent by performing the process of the invention, wherein a compound that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate compound for the treatment of diabetes;

(b) optionally, isolating the candidate agent;

(c) optionally, providing the name or structure of the candidate agent;

(d) optionally, providing the candidate agent; and

(e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

Suitable agents and methods for determining their affect on GLUT4 translocation are described *supra*. Additionally, methods for inducing insulin resistance in a cell are described *supra*. For example, the cell is treated with insulin in the absence of serum for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell.

For example, the agent is formulated into a pharmaceutical formulation. Formulation of a pharmaceutical compound will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition comprising the identified modulator to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils, for instance. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers and the like (See, generally, Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Co., Pa., 1985). For inhalation, the agent can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

Furthermore, where the agent is a protein or peptide or antibody or fragment thereof, the agent can be administered via *in vivo* expression of the recombinant protein. *In vivo* expression can be accomplished via somatic cell expression according to suitable methods (see, e.g. U.S. Pat. No. 5,399,346). In this embodiment, nucleic acid encoding the protein can be incorporated into a retroviral, adenoviral or other suitable vector (preferably, a replication deficient infectious vector) for delivery, or can be introduced into a transfected or transformed host cell capable of expressing the protein for delivery. In the latter embodiment, the cells can be implanted (alone or in a barrier device), injected or otherwise introduced in an amount effective to express the protein in a therapeutically effective amount.

The pH and exact concentration of the various components the formulation suitable for administration to the animal are adjusted according to routine skills in the art.

Following determination of an agent using a method described herein, the agent is additionally tested *in vivo*. For example, a candidate agent for the treatment of a mouse

or rat model of NIDDM. For example, a mouse model is a mouse, such as for example a Cpe^{fat} mouse, a Lep^{ob} mouse, a $Lepr^{ob}$ mouse or a tub mouse (all available from Jackson Laboratories). Alternative models of NIDDM include, for example, the tallyho mouse (Kim *et al., Genomics 74*: 273-286, 2001) or the OLETF rat (Watanabe *et al., Genomics 58*: 233-239). Such models are useful for, for example, determining the toxicity of a compound and/or the efficacy of a compound (e.g., the level or amount of the compound required for treatment).

The present invention is further described with reference to the following non-limiting examples

EXAMPLE 1
GENERATION AND EXPRESSION OF A LABELED GLUT4 PROTEIN

A HA-tagged GLUT4 protein was produced essentially as described in Quon *et al., Proc. Natl. Acad. Sci USA 94*: 5587-5591, 1994. Essentially, the cDNA encoding GLUT4 was digested with *Sau*I and a double stranded oligonucleotide was inserted by ligation. The double stranded oligonucleotide was formed by hybridizing two oligonucleotides one comprising the sequence TGAGATCGATTATCCTTATGATGTTCCTGATTATGG (SEQ ID NO: 63) and the other TCA GCA TAA TCA GGA ACA TCA TAA GGA TAA TCG ATC (SEQ ID NO: 64). The inserted nucleic acid encodes a HA tag between amino acids 67 and 68 in the first exofacial loop of GLUT4 (SEQ ID NO: 4). This gene construct was inserted into the vector pBABE (Pear *et al. Proc. Natl Acad. Sci. U.S.A. 90:* 8392-8396 1993). The polypeptide encoded by this protein is shown schematically in Figure 1A.

Additional gene constructs were generated comprising a nucleic acid encoding mutant forms of GLUT4 (these constructs encoded the TAIL mutant of GLUT4 (SEQ ID NO: 5), the L489,490A mutant of GLUT4 (SEQ ID NO: 7) and the F5A mutant of GLUT4 (SEQ NO: 9), each tagged with a HA tag), comprising a HA tag in the first extracellular domain of the protein, essentially as described in Piper *et al, The Journal of Cell Biology, 121(6)*:1221-1232, 1993, Marsh *et al, JCB, 130(5)*: 1081-1091, 1995, Shewan *et al. Biochem. J. 350:* 99-107, 2000 and Shewan *et a, Mol. Biol. Of Cell, 14*: 973-986, 2003. The proteins encoded by these nucleic acids are schematically represented in Figure 1B.

Retroviral stocks of each of the constructs were produced using the method described in Pear *et al. Proc. Natl Acad. Sci. U.S.A. 90:* 8392-8396 1993. To generate 3T3-L1 adipocytes stably expressing the each construct 3T3-L1 fibroblasts (plated at a density of 5 x 10^5/ 100mm plate 16 h beforehand) were infected with the relevant virus for 3-5h in the presence of 4μg/ml Polybrene (Sigma). After a 48h recovery period, infected cells were then selected in DMEM containing 10% FCS and supplemented with 2μg/ml puromycin (Sigma).

3T3-L1 fibroblasts up to passage 20 were cultured in high glucose DMEM supplemented with 10% heat-inactivated new born calf serum (NCS) at 37°C in 5% CO2. For differentiation into adipocytes, fibroblasts were cultured in DMEM/NCS for

up to one or two days post-confluence, after which the cells were cultured for three days in DMEM containing 10% heat-inactivated fetal bovine serum (FBS), 350 nM insulin, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 250 nM dexamethasone, 400 nM biotin and for three days in DMEM containing 10% FBS and 350 nM insulin. After differentiation, adipocytes were maintained in DMEM supplemented with 10% FBS. Adipocytes were used for experiments 8 to 11 days after the onset of differentiation and the medium was renewed two or three days prior to each experiment. For culturing in gelatin-coated 96 well plates, cells were seeded at a 1:1 cell surface ratio and differentiation was initiated four days post-seeding.

To determine expression of the constructs transduced cells were studied suing immunofluorescence. Cells were stained for either the HA tag (Covance, Berkeley, CA, USA) or anti-GLUT4 (Martin *et al., J. Cell Biol. 134*: 625-635, 1994). As shown in Figure 1D approximately 90% of cells expressed the recombinant HA-GLUT4.

Steady state labeling of unstimulated cells revealed a predominant perinuclear GLUT4 localization in fibroblasts with low levels of GLUT4 in small peripheral vesicles. GLUT4 TAIL was more concentrated in peripheral vesicles compared to wild-type GLUT4 when expressed in fibroblasts (Fig. 1G).

Expression levels of the expression of the recombinant forms of GLUT4 was then determined using immunoblotting. Confluent 3T3-L1 fibroblasts and 3T3-L1 adipocytes at day 8 of differentiation were serum-starved for 2 h and lysed in PBS containing 1% Triton X-100, 1 mM EDTA, 1 mM PMSF, 10μg/ml aprotinin and 10μg/ml leupeptin. Equal amounts of protein were subjected to SDS- PAGE and transferred to PVDF membrane. Membranes were incubated with the indicated antibodies. HRP-conjugated secondary antibodies were visualized using ECL reagent (Pierce, Rockford, IL) and a 16 bit camera-based imager (VersaDoc 5000; Bio-Rad, Regents Park, Australia). For quantitation, a serial dilution of a control sample was run on the same SDS-PAGE gel and Quantity One software (Bio-Rad, Regents Park, Australia) was used for analysis. An anti-HA immunoblot was used to determine the relative expression of GLUT4 TAIL as this GLUT4 molecule was not recognized by the anti-GLUT4 antibody.

There was a modest level of overexpression (Fig. 1E and 1F), making it unlikely that GLUT4 localization was disturbed due to saturation of the cellular trafficking machinery.

EXAMPLE 2
GENERATION OF AN ASSAY TO DETERMINE THE LOCALIZATION OF GLUT4

2.1 Methods

Retrovirally-transduced fibroblasts expressing HA-tagged GLUT 4 or a mutant therof were differentiated into adipocytes essentially as described above. These adipocytes were then subcultured for 30 hours. Insulin was then added at different time points, after which the cells were fixed in 3% formaldehyde. After washing and quenching with 50 mM glycine, cells were incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of GLUT4 at the plasma membrane (PM) or the total cellular GLUT4 content, respectively. Cells were incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells were incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize all cells. Cells were incubated for 60 min with saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 μg/ml) and ALEXA594-conjugated WGA (10 μg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) was measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT4 at the PM was calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

2.2 Results

To determine the extent of insulin-induced GLUT4 translocation using the assay described *supra*, HA-GLUT4-expressing 3T3-L1 adipocytes grown in 96 well plates were incubated for 2 h in the absence of serum, whereafter 200 nM insulin was added at various time points and cell surface levels of HA-GLUT4 were analysed by indirect immunofluorescence labeling (Fig. 2B). Saturating levels of anti-HA and secondary antibodies were used to ensure that substantially all HA-GLUT4 molecules were labeled. A non-relevant antibody was used at the same concentration to determine the

non-specific binding of the anti-HA antibody. Insulin stimulated the appearance of HA-GLUT4 at the PM with a half-time of about 2.5 min reaching a plateau by 12 min, which was maintained for at least 60 min. No specific anti-HA labeling was detected in non-infected cells (Fig. 2A). Expressing the amount of specific fluorescence at the PM as a percentage of the total specific fluorescence revealed that insulin increased the level of GLUT4 at the PM from a basal value of 4% up to 34% (Fig. 2C) and this effect was inhibited by wortmannin (Fig. 2D).

EXAMPLE 3

Insulin induced translocation of GLUT4 in 3T3-L1 fibroblasts and adipocytes

In fibroblasts, insulin induced the translocation of wild-type GLUT4 and each of the GLUT4 mutants to the PM (Fig. 3). The maximum level of surface GLUT4 was reached after 6 min of insulin stimulation, representing a 5-fold increase above that observed in non-stimulated cells, followed by a rapid reduction. The PM level of the GLUT4 F5A mutant was slightly higher than that of the other GLUT4 molecules in insulin-stimulated fibroblasts. In adipocytes we observed an ~8-fold increase in cell surface GLUT4 levels in response to insulin stimulation. Neither wild-type GLUT4 nor any of the GLUT4 mutants showed an overshoot as was observed in fibroblasts. The GLUT4 TAIL mutant showed translocation characteristics similar to those of GLUT4 WT, although cell surface levels in both the absence and presence of insulin were increased by approximately 5%, in accordance with previous studies (Shewan *et al., Mol. Biol. Cell 14*: 973-986, 2003). The PM levels of both the L489,490A and F5A mutants were significantly higher than those of GLUT4 WT, both in the absence and presence of insulin.

EXAMPLE 4

GLUT4 internalization and recycling in 3T3-L1 adipocytes

4.1 Methods

For single cycle internalization experiments cells were stimulated for 20 min with 200 nM insulin after starvation and washed on ice with ice-cold DMEM containing 20 mM HEPES pH 7.4 and 0.2% BSA. Cells were incubated with 100 nM wortmannin or 200 nM insulin and either anti-HA (25 μg/ml) or non-relevant antibody (MOPC21) in DMEM/HEPES/BSA for 1 h on ice. Wortmannin was added to abolish insulin signalling. This drug has no direct effect on GLUT4 internalization in adipocytes

(Malide and Cushman *J. Cell Sci. 110*: 2795-2806) and has previously been used to study GLUT4 internalization (Al-Hasani *et al, J. Biol. Chem. 273*: 17504-17510). Cells were washed extensively, then either 100 nM wortmannin or 200 nM insulin in DMEM/HEPES/BSA was added. The plate was then transferred to 37°C and at different times, formaldehyde was added to the wells to a concentration of 3%. After 5 min the formaldehyde was washed away and residual amounts were quenched. The cells were incubated for 20 min with 5% NSS in the absence of saponin, labeled with ALEXA488-conjugated goat-anti-mouse antibody and ALEXA594-conjugated WGA, washed and analysed as described above.

For continuous antibody uptake experiments, cells were incubated for 20 min with or without insulin, whereafter anti-HA (50 μ g/ml) or non-relevant antibody was added. Cells that were used to determine the total amount of HA-GLUT4 were not incubated with antibody during this 37°C incubation. After incubation, the cells were fixed and quenched as described above, and incubated for 20 min with 5% NSS and 0.1% saponin. Cells that were used to determine the total cellular amount of HA-GLUT4 were incubated for 60 min with anti-HA antibody or control antibody in PBS containing 2% NSS. All other cells were incubated with 2% NSS without antibody. Subsequently, the cells were incubated with ALEXA488-conjugated goat-anti-mouse antibody and ALEXA594-conjugated WGA, washed and analysed. The amount of specific anti-HA uptake was expressed as a percentage of total cellular immuno-reactive HA-GLUT4.

4.2 Analysis of GLUT4 internalization in 3T3-L1 adipocytes

GLUT4 WT molecules that were labeled with anti-HA antibody on ice were rapidly cleared from the cell surface as indicated by the disappearance of GLUT4 at early time points after transfer of the cells from ice to 37°C (Fig. 4). After approximately 5 min the level of GLUT4 at the PM reached steady state in the presence but not in the absence of insulin, indicating recycling of GLUT4 back to the PM in insulin-stimulated cells. Our data indicated that after 2 min at 37°C ~50% of both GLUT4 WT and GLUT4 TAIL had disappeared from the PM. Importantly, this internalization rate was unaffected by insulin, consistent with previous studies (Satoh *et al., J. Biol. Chem. 268*: 17820-17829, 1993). The internalization rates for the L489,490A and F5A mutants were decreased by 30 and 45%, respectively (Fig. 4).

4.3 Anti-HA antibody uptake by HA-GLUT4-expressing fibroblasts and adipocytes

To analyze the exchange of GLUT4 with the cell surface under steady state conditions, studies were performed in which live cells were incubated with anti-HA antibody at 37°C (Fig. 5). To ascertain that the anti-HA antibody did not affect the intracellular trafficking of HA-GLUT4, control experiments were performed in which insulin-induced translocation of anti-HA-bound HA-GLUT4 was studied. 3T3-L1 adipocytes expressing HA-GLUT4 WT were stimulated for 2 h with 200 nM insulin in the presence of anti-HA antibody, washed extensively, incubated for 2 h without insulin and anti-HA, and incubated for a further 20 min in the absence (Fig. 5C) or presence (Fig. 5D) of 200 nM insulin. The cells showed insulin-induced redistribution of anti-HA-bound HA-GLUT4 from intracellular compartments to the PM that was indistinguishable from translocation of HA-GLUT4 that had not been pre-labeled with antibody (Fig. 5A and 5B), indicating that the anti-HA antibody had no significant effect on GLUT4 trafficking.

For quantification of anti-HA antibody uptake, cells were preincubated for 20 min in the presence or absence of insulin after which anti-HA antibody or control antibody was added for various times (Fig. 5E). Antibody uptake was determined by labeling cells with fluorescent secondary antibody after fixation. Antibody uptake was expressed as a percentage of post-fixation anti-HA labeling.

Several observations were made from these studies. Firstly, there was a profound difference in recycling kinetics for HA-GLUT4 between fibroblasts and adipocytes in the absence of insulin. Whereas in fibroblasts a significant portion of the GLUT4 molecules recycled between intracellular compartments and the PM in the absence of insulin (~50% after 60 min), this was not the case in adipocytes with only ~10% of the entire GLUT4 pool labeled after 3 h. A similar percentage of GLUT4 was labeled after 6 h (not shown). Recycling of HA-GLUT4 in the presence of insulin was similar for fibroblasts and adipocytes. Secondly, the recycling rate of HA-GLUT4 TAIL in non-stimulated adipocytes was significantly higher than that observed for GLUT4 WT.

Thirdly, both of the internalization mutants showed a minor increase in basal anti-HA uptake and no difference in uptake during insulin stimulation compared with GLUT4 WT. Finally, it was noted that even with maximum insulin stimulation a small but significant pool of GLUT4 did not exchange with the cell surface under steady state conditions. The size of this pool was similar between fibroblasts and adipocytes and for

the different GLUT4 mutants suggesting that it represents a pool of GLUT4 that is segregated from the insulin responsive pool.

To study this non-recycling GLUT4 pool in adipocytes, 3T3-L1 adipocytes expressing HA-GLUT4 WT were incubated at 37°C in the continuous presence of anti-HA antibody. Cells were incubated with or without 200 nM insulin for 20 min, after which anti-HA antibody was added in the continued presence or absence of insulin. Cells were incubated further for up to 180 min, fixed, permeabilized, and incubated with fluorescent secondary antibody. The level of anti-HA antibody taken up by the cells was then expressed as a percentage of total post-fixation anti-HA labeling of permeabilized cells. As shown in Fig 6A, only approximately 30% of the HA-GLUT4 detected in a cell is labeled in the insulin induced cells. This suggests that approximately 30% of the HA-GLUT4 expressed in the cell did not translocate to the membrane during the experiment.

The cells that were used to determine the 100% value of HA-GLUT4 that recycled to the plasma membrane were incubated again with fixative after the post-fixation anti-HA immunolabeling. As shown in Fig 6B fixation of the anti-HA antibody appeared not to change the affinity of the secondary antibody and therefore did appear not cause the 30% of difference in labeling.

Cells were again incubated with anti-HA after fixation without permeabilization. As shown in Fig 6C the 30% of HA-GLUT4 that cannot be labeled with antibody during the 37°C incubation is not present at the cell surface. Furthermore, cells were incubated again with the anti-HA antibody after fixation and permeabilization. In this case, 100% of GLUT4 was labeled, indicating that the 30% of HA-GLUT4 that cannot be labeled during the continuous antibody uptake is not unable to bind antibody but remains intracellular during the antibody uptake incubation.

To determine whether or not the antibody concentration used limited the level of HA-GLUT4 detected in a cell, cells were incubated for 3 h in the presence of insulin with various concentrations of anti-HA (in this regard, the standard concentration used was 50 mg/ml). As shown in Figure 6E antibody concentration during the antibody incubation appeared not to be limiting with comparable levels of HA-GLUT4 being detected with various concentrations of anti-HA antibody.

To determine whether or not the unlabeled HA-GLUT4 was still in the process of synthesis or part of the biosynthetic tract cells were incubated with 10 mg/ml cycloheximide for 2 h prior to the addition of antibody. As shown in Figure 6F 30% of GLUT4 could not be labeled, suggesting that the non-labeled GLUT4 pool is not part of the biosynthetic tract.

To determine the effect of endosomal pH on the binding of anti-HA antibody to HA-GLUT4 was determined. Cells were incubated for 30 min at 37°C in hypertonic medium (0.45 M sucrose, pH 7.4), on ice with antibody in the same medium, and at 37°C in hypertonic buffer at pH 7.4 or pH 5.5 in the absence of antibody. Release of antibody from the plasma membrane at neutral or endosomal pH was determined by incubating fixed non-permeabilized cells with fluorescent secondary antibody. As shown in Figure 6G, endosomal pH did not induce the release of the anti-HA antibody from the HA-tag.

The effect of long-term insulin treatment on the amount of cell surface HA-GLUT4 levels was also determined. In this regard, cells were incubated for various times with 200 nM insulin and cell surface GLUT4 levels were determined as described *supra*. As shown in Figure 6H, insulin did not drastically down-regulate cell surface GLUT4 levels, indicating that insulin-induced down-regulation of GLUT4 at the PM did not account for the limited HA-GLUT4 labeling during the continuous antibody uptake.

The recycling kinetics of HA-GLUT4 was studied at different stages throughout fibroblast differentiation (Fig. 7). In parallel, antibody uptake was analysed by immunofluorescence confocal microscopy (Fig. 7, left microscopy panels) as well as endogenous GLUT4 labeling and lipid droplet content in non-infected cells (Fig. 7, right microscopy panels).

There was a progressive decline in antibody uptake between days 0 and 4 of differentiation. Expression of endogenous GLUT4 and lipid droplet formation were initially detected at day 3 when antibody uptake by non-stimulated cells had already decreased by 85% (compared with 100% at day 4). The final reduction in basal anti-HA uptake, between day 3 and 4, coincided with a massive growth of the cells (Fig. 7, right bottom microscopy panels).

The results attained suggest that only part of the intracellular GLUT4 pool may be released into the cell surface recycling system as opposed to reduced trafficking kinetics of the entire intracellular GLUT4 pool. To test this recycling studies were performed at different doses of insulin (Fig. 8). These studies revealed that the size of the recycling pool of GLUT4 was incrementally increased with increasing doses of insulin.

This phenomenon was evident for both GLUT4 WT and GLUT4 TAIL, although insulin had a less profound effect on GLUT4 TAIL due to its elevated levels in the recycling pathway in the basal state (Fig. 5 and 8B). Measurement of cell surface levels of HA-GLUT4 at the different insulin doses revealed that the insulin dose response curves for translocation of both GLUT4 WT and TAIL were similar, despite major differences in their basal recycling properties (Fig. 8B).

To rule out the possibility that this incremental effect of insulin on entry of GLUT4 into the cell surface recycling system might reflect intrinsic differences in insulin sensitivity between individual cells within the culture the dose response relationship in antibody uptake in individual cells using immunofluorescence microscopy was examined. As indicated in Fig. 8C the response among different cells was highly homogeneous such that at low doses of insulin most cells exhibited a low level of antibody uptake and at higher doses there was a uniform rather than a heterogeneous increase in antibody uptake.

EXAMPLE 5

Development of a high-throughput assay for determining GLUT4 translocation

To determine the efficacy of a high throughput assay for analysing the level of translocation of a labeled membrane transport protein HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 384 well plates or first grown in Petri dishes and then relocated into the 384 well plates. An incubation period of 2 hours was observed after which 200nM insulin exposure was used for the indicated periods of time. For each time point the percentage of labeled GLUT4 (compared to the level of labeled GLUT4 following cell permeabilization) at the plasma membrane was calculated. As shown in Figure 9 approximately equal levels of GLUT4 translocation was observed in both

sample types. Accordingly, these results show the efficacy of a 384 high-throughput method for analysing GLUT4 translocation.

EXAMPLE 6

The effect of amino acid concentration on GLUT4 translocation

HA-GLUT4 expressing adipocytes were serum starved for 2 hours in Krebs Ringer Phosphate (KRP) buffer or in the same buffer supplemented with amino acid concentrations used in Dulbecco's modified eagle medium of Gibco (2x amino acids) or with half of the amino acid concentration (1x amino acids) respectively. Cells were then stimulated with 200nM insulin essentially as described above and the percentage of HA-GLUT4 WT translocated to the membrane determined as described *supra*. As shown in Fig. 10 the concentration of amino acids in the medium in which cells were incubated influenced the level of GLUT4 translocated to the plasma membrane.

EXAMPLE 7

Inducing GLUT4 translocation to the plasma membrane

3T3-L1 adipocytes expressing HA-GLUT4 WT were serum starved for 2 hours at 37oC. Following 20 minutes insulin stimulation with 200nM insulin, cells were incubated for additional 2 hours in serum free medium supplemented with 0.2% BSA and 0.3 or 0.6M sucrose. After post-fixation anti-HA immunolabeling the level of cell surface HA-GLUT4 levels was determined as a percentage of total HA-GLUT4 detected after cell lysis. As shown in Fig. 11, sucrose dramatically increases the level of HA-GLUT4 translocated to the plasma membrane of a cell. Furthermore, increasing concentrations of sucrose induce more GLUT4 to translocate to the plasma membrane in the presence of reduced levels of insulin.

EXAMPLE 8

Development of a model of insulin resistance

3T3-L1 adipocytes retrovirally infected with GLUT4 (described in Example 1) were incubated 24 hours or 48 hours either with 600nM insulin or with medium alone. After this chronic insulin stimulation (as indicated in Figure 10) at 37°C in a CO_2 incubator, cells were washed and 200 nM insulin was added for additional 10 or 30 minutes. Cell surface levels of HA-GLUT4 were measured using the fluorescence based assay

described *supra* and expressed as a percentage of total HA-GLUT4 detected in the cell. The experiment was also performed with the HA-GLUT4 TAIL mutant.

As shown in Figure 12A the level of GLUT4 at the plasma membrane of cells incubated in the presence of serum was dramatically increased following 24h incubation in the presence of insulin. However, this effect was suppressed following 48h incubation in the presence of insulin.

A dramatically different effect was observed in cells incubated in the absence of serum (either -serum or KRP). The levels of GLUT4 translocation observed were little more than basal levels (i.e. cells in the absence of insulin). These results indicate that the cells were resistant to insulin induced GLUT 4 translocation. This assay represents an attractive model of insulin resistance for, for example, screening for agents for treating disorders characterised by insulin resistance.

As shown in Figure 12B similar results were attained with the HA-GLUT4 TAIL mutant.

Furthermore, as shown in Figure 13 wortmannin was shown to have little effect on the translocation of HA-GLUT4 in the presence of serum either following an acute or chronic exposure to insulin. HA-GLUT4 expressing 3T3-L1 adipocytes were grown in 96 well plates, incubated for 2 hours or overnight in medium supplemented with 10% fetal calf serum or no serum. 200nM insulin in case of acute stimulation and 600nM insulin in case of chronic stimulation have been used. After overnight stimulation cells were washed and 200nM fresh insulin was added for 10 or 30 min.

However, following an acute exposure to insulin, wortmannin was able to reduce levels of HA-GLUT4 translocation in cells incubated in the absence of insulin. Following a chronic exposure of the cells to insulin wortmannin did not appear to significantly alter the levels of GLUT4 translocated to the plasma membrane.

EXAMPLE 9

Screening a natural product library to determine an enhancer of GLUT4 translocation

HA-GLUT4 expressing 3T3-L1 adipocytes are grown in 384 well plates essentially as described in Example 5. Cells are then incubated 24 hours with 600nM insulin in the

absence of serum. After this chronic insulin stimulation at 37°C in a CO_2 incubator cells are incubated in the presence of a compound from a natural product library, such as, for example, the plant extract library from TimTec (Newark, USA). 200 nM insulin is then added for an additional 10 or 30 minutes to each well. Cell surface levels of HA-GLUT4 is measured using the fluorescence based assay described *supra* and expressed as a percentage of total HA-GLUT4 detected in the cell. Results are also normalized for cell number using WGA, essentially as described in Example 2.

Samples are analysed to determine those natural products that are capable of inducing HA-GLUT4 translocation to the plasma membrane to a degree similar to that observed in a cell incubated in the presence of both serum and insulin (i.e. a positive control).

Cells cultured in parallel are also assayed using trypan blue exclusion to determine those natural products that are toxic to cells. Following incubation of the cells in the presence or absence (control) of the natural products, cells are treated with 1% trypan blue. The number of cells that have taken up the trypan blue stain in each treatment group is expressed as a percentage of the number of cells that have taken up the trypan blue stain in the control samples. Those compounds that significantly reduce the number of viable cells are considered to be at least partially toxic to a cell.

Compounds that enhance GLUT4 translocation without significantly reducing viability are then assessed using the assays *supra* to determine the concentration at which translocation is maximally enhanced without affecting cell viability.

EXAMPLE 10

In vivo analysis of an enhancer of GLUT4 translocation

Male C57BL/KS-Lep^{db} (*db/db*) and nondiabetic littermate mice (The Jackson Laboratory) are obtained at 7-8 weeks of age and housed in 12 hr of light per day at 21-23°C and 40-60% humidity. All experiments begin at 10 weeks of age. A compound determined in Example 9 is administered by sub cutaneous injection. For glucose tolerance testing, all animals were fasted for 16-18 hr before gavaging with a standard glucose bolus, as outlined Tonra *et al., Diabetes 48*: 588-594, 1999. Animals are then anesthetized and a bolus of insulin (1 unit) administered through the jugular vein; 2 or 10 min later, the liver is rapidly removed and frozen at -80°C until processed.

Serum samples are taken between 1000 and 1200 hours and analyzed for glucose, triglycerides, and cholesterol with the Monarch blood chemistry analyzer (Instrumentation Laboratory, Lexington, MA). NEFA are analyzed with a diagnostic kit (Wako Chemical, Osaka) and insulin levels by ELISA (Linco Research Immunoassay, St. Charles, MO). For analysis of endogenous lipids, frozen sections of liver are mounted on glass slides and stained with oil red O. Liver glycogen is measured from frozen tissue by assaying for glucose after amyloglucosidase digestion with a correction for nonglycogen glucose (Tonra *et al., Diabetes 48*: 588-594, 1999).

Using these assays, mice are then assessed to determine hyperinsulinemia, hyperglycemia and glucose tolerance essentially as described in Sleeman *et al., Proc Natl Acad Sci U S A. 100*:14297-14302, 2003. For example, serum glucose and insulin levels are determined.

EXAMPLE 11

An assay to determine a suppressor of GLUT4 translocation

HA-GLUT4 expressing 3T3-L1 adipocytes are grown in 384 well plates essentially as described in Example 5. Cells are then incubated with a compound from the natural product library *supra* and then 200nM insulin. The level of HA-GLUT4 translocated to the palsma membrane is then measured.

Briefly, cells are fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of GLUT4 at the plasma membrane (PM) or the total cellular GLUT4 content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize all cells. Cells are incubated for 60 min with saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 μg/ml) and ALEXA594-conjugated WGA (10 μg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT4 at

the PM is calculated for each compound. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

As a positive control the K+/H+ exchanger, nigericin, is used. Nigericin is known to inhibit insulin mediated GLUT4 translocation Chu *et al., J Cell Biochem. 2002;85*:83-91. The level of translocation of HA-GLUT4 for each natural compound is compared to that for nigericin and compounds with equal or greater inhibitory activity are selected.

In parallel cultures, the toxicity of each of the natural products is also assessed. Cell viability for each of the compounds tested is assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega) essentially according to manufacturer's instructions. Compounds that do not significantly reduce cell viability are selected for further analysis.

The compounds selected are then screened using the HA-GLUT4 translocation assay and the CellTiter-Glo® Luminescent Cell Viability Assay to determine the concentration at which each compound shows maximum activity without significantly reducing cell viability.

EXAMPLE 12
A model for GLUT1 translocation

12.1 Vector construction

A human GLUT1 cDNA containing an Hemagglutinin epitope tag in its first exofacial loop was kindly provided in the pCIS2 expression vector by the Al-Hasani Lab.

HA-GLUT1 is then excised from this pCIS2 vector by *Nde*I and *Kpn*I digestion and subcloned into the pOK12 plasmid . Following digestion with *Nde*I and *Kpn*I, this reporter GLUT1 gene tagged with HA is then excised from pOK12 plasmid as a 1.8 kb *Cla*I/*Xba*I fragment and subcloned into pBluescript plasmid digested with *Cla*I and *Xba*I. Following subcloning, the HA-Glut1 fragment is excised from pBluescript by *BstX*I and *Sal*I digestion and directionally cloned into pBABE retrovirus expression vector digested with *BstX*I and *Sal*I, thus generating the HA-GLUT1..

12.2 retrovirus production and transduction

Retroviral stocks of the construct is produced using the method described in Pear *et al. Proc. Natl Acad. Sci. U.S.A. 90:* 8392-8396 1993. To generate C2C12 myoblast cells stably expressing the expression construct C2C12 were infected with the relevant virus for 3-5h in the presence of 4μg/ml Polybrene (Sigma). After a 48h recovery period, infected cells are then selected in DMEM containing 10% FCS and supplemented with 2μg/ml puromycin (Sigma).

Transduced myoblasts are seeded in proliferation medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS) at a density of 12,000 cells per cm^2 and grown for 48 h to confluency. Cells are washed once with serum-free medium and induced to fuse in medium containing 2% horse serum (differentiation medium).

12.3 Analysis of translocation of HA-GLUT1 in differentiated C2C12 cells

Retrovirally-transduced differentiated C2C12 cells expressing HA-tagged GLUT1 are subcultured for 30 hours. Insulin is then added at different time points, after which the cells are fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of HA-GLUT1 at the plasma membrane (PM) or the total cellular HA-GLUT1 content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody (mouse IgG MOPC21) in PBS containing 2% NSS. After extensive washing, the cells are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize the cells. Cells are incubated for 60 min with saturating concentrations of ALEXA488-conjugated goat-anti-mouse antibody (20 μg/ml) and ALEXA594-conjugated WGA (10 μg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of GLUT1 at the PM is calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

As a positive control a sample of cells are also incubated in the presence of Dehydroepiandrosterone (DHEA). DHEA has been previously shown to enhance levels of GLUT1 at the plasma membrane of a cell (Perrini *et al., Diabetes 53*:41-52, 2004).

EXAMPLE 13

A model to determine the effect of a CFTR mutation on CFTR translocation

The coding region of the CFTR gene (SEQ ID NO: 35) is isolated using methods essentially as described in Rommens *et al., Proc. Natl. Acad. Sci. USA 88*: 7500-7504, 1990. A double stranded oligonucleotide encoding HA tag is then inserted so as to encode the tag at the N terminus of the protein. The N-terminus of the CFTR is predicted to be an extracellular domain of the protein.

A vector comprising nucleic acid encoding the ΔF508 mutant of CFTR (SEQ ID NO: 62) is produced essentially as described in Tabacharani *et al., Nature, 352*: 628-632, 1991. The nucleic acid encoding the mutant CFTR is then modified to insert a double stranded oligonucleotide encoding HA tag is then inserted so as to encode the tag at the N terminus of the protein.

Each of the modified constructs is then cloned into the pBABE retroviral vector.

Retroviral stocks of each of the constructs are then produced using the method described in Pear *et al. Proc. Natl Acad. Sci. U.S.A. 90:* 8392-8396 1993. To generate COS cells stably expressing the expression construct COS were infected with the relevant virus for 3-5h in the presence of 4μg/ml Polybrene (Sigma). After a 48h recovery period, infected cells are then selected in DMEM containing 10% FCS and supplemented with 2μg/ml puromycin (Sigma).

The level of plasma membrane associated HA-CFTR or HA-CFTR-ΔF508 is then determined. Briefly, Retrovirally-transduced cells expressing HA-tagged CFTR or CFTR-ΔF508 are subcultured for 30 hours. Cells are then fixed in 3% formaldehyde. After quenching with 50 mM glycine, cells are incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyse the level of HA-labeled CFTR or mutant thereof at the plasma membrane (PM) or the total cellular HA-CFTR or CFTR-ΔF508 content, respectively. Cells are incubated for 60 min with a saturating concentration of either an antibody directed against the HA tag or a control non-relevant antibody in PBS containing 2% NSS. After extensive washing, the cells are incubated for 20 min with 5% NSS in the presence or absence of 0.1% saponin to permeabilize the cells. Cells are incubated for 60 min with saturating concentrations of

ALEXA488-conjugated goat-anti-mouse antibody (20 μg/ml) and ALEXA594-conjugated WGA (10 μg/ml) in PBS containing 2% NSS. After washing, fluorescence (emm 485/exc 520 and emm 544/exc 630) is measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany). The percentage of CFTR or CFTR-ΔF508 at the PM is calculated for each condition. ALEXA594-WGA fluorescence was used to correct for variation in cell density in each well.

By comparing the level of HA-CFTR at the plasma membrane compared to the level of HA-CFTR-ΔF508 translocated to the plasma membrane, the effect of the ΔF508 mutation on translocation is determined.

We claim:

1. A process for determining the level of a membrane transport protein translocated to the plasma membrane of a cell, said method comprising:

(a) determining the level of a membrane transport protein at the plasma membrane of the cell using a method comprising:

(i) contacting the cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein at the plasma membrane of the cell; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) (i) permeabilizing or disrupting the plasma membrane of a cell and contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein; and

(c) comparing the level of ligand determined at (a) (ii) and (b) (ii) to determine the level of the membrane transport protein at the plasma membrane relative to the level of the membrane transport protein inside the cell.

2. The process according to claim 1 wherein the membrane transport protein is a glucose transport (GLUT) protein.

3. The process according to claim 2 wherein the membrane transport protein is GLUT4.

4. The process according to claim 3 wherein the GLUT4 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.

5. The process according to claim 2 wherein the membrane transport protein is GLUT1.

6. The process according to claim 5 wherein the GLUT1 comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 12.

7. The process according to claim 1 wherein the membrane transport protein is a mutant membrane transport protein having a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein.

8. The process according to claim 7 wherein the reduced rate of recycling or transporter internalization of the mutant membrane transport protein increases the level of the mutant membrane transport protein at the plasma membrane of a cell compared to the level of a wild-type form of the membrane transport protein.

9. The process according to claim 8 wherein the mutant protein is a mutant GLUT4 protein.

10. The process according to claim 10 wherein the mutant GLUT4 protein comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9.

11. The process according to claim 1 wherein the membrane transport protein is labeled to facilitate binding of the ligand to the membrane transport protein.

12. The process according to claim 11 wherein the label comprises one or more copies of a peptide, polypeptide or protein that is heterologous to the membrane transport protein.

13. The process according to claim 12 wherein the label comprises one or more copies of a peptide, polypeptide or protein selected from the group consisting of influenza virus hemagglutinin (HA) (SEQ ID NO: 15), Simian Virus 5 (V5) (SEQ ID NO: 16), polyhistidine (SEQ ID NO: 17), c-myc (SEQ ID NO: 18), FLAG (SEQ ID NO: 19), GST (SEQ ID NO: 22), MBP (SEQ ID NO: 23), GAL4 (SEQ ID NO: 24), β-galactosidase (SEQ ID NO: 25), enhanced green fluorescence protein (eGFP) (SEQ ID NO: 26), yellow fluorescent protein (SEQ ID NO: 27), soluble modified blue fluorescent protein (SEQ ID NO: 28), soluble-modified red-

shifted green fluorescent protein (SEQ ID NO: 29), cyan fluorescent protein (SEQ ID NO: 30) , biotin, strepavidin, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 20, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 21, a peptide comprising the amino acid sequence set forth in SEQ ID NO: 31 and mixtures thereof.

14. The process according to claim 13 wherein the label comprises influenza virus hemagglutinin (HA) (SEQ ID NO: 15).

15. The process according to claim 12 wherein the label is positioned within an extracellular domain of the membrane transport protein.

16. The process according to claim 15 wherein the label is positioned within the first extracellular domain of a GLUT protein or a mutant thereof.

17. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT4 protein or a mutant GLUT4 protein that comprises an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

18. The process according to claim 12 wherein the labeled membrane transport protein is a GLUT1 protein that comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 13.

19. The process according to claim 1 wherein the cell is a eukaryotic cell.

20. The process according to claim 19 wherein the cell is a mammalian cell

21. The process according to claim 20 wherein the cell is a cell selected from the group consisting of a 3T3-L1 fibroblast cell, a 3T3-L1 adipocyte cell and a C2C12 cell.

22. The process according to claim 1 wherein the ligand capable of binding to the membrane transport protein is an antibody.

23. The process according to claim 22 wherein the antibody is a monoclonal antibody.

24. The process according to claim 23 wherein the monoclonal antibody is an anti-hemagglutinin (HA) tag antibody capable of binding to an amino acid sequence set forth in SEQ ID NO: 15.

25. The process according to any one of claims 22 to 24 wherein the antibody is labeled with a detectable marker selected from the group consisting of an enzyme label, a radiolabel and a fluorescent label.

26. The process according to any one of claims 23 to 25 wherein the antibody is labeled with a fluorescent label.

27. The process according to claim 1 wherein the plasma membrane is permeablilized or disrupted by contacting the plasma membrane with an agent that permeabilizes or disrupts a membrane for a time and under conditions sufficient for permeabilization or disruption to occur.

28. The process according to claim 27 wherein the agent that permeabilizes or disrupts a membrane is selected from the group consisting of saponin, n-octyl-glucopyranoside, n-Dodecyl β-D-maltoside, N-Dodecanoyl-N-methylglycine sodium salt, hexadecyltrimethylammonium bromide, deoxycholate, a non-ionic detergent, streptolysin-O (SEQ ID NO: 32), α-hemolysin (SEQ ID NO: 33), tetanolysin (SEQ ID NO: 34) and mixtures thereof.

29. The process according to claim 28 wherein the agent that permeabilizes or disrupts the membrane is saponin.

30. The process according to claim 1 wherein the level of the ligand bound to the membrane transport protein is determined by a process comprising contacting the ligand with an antibody that specifically binds to the ligand for a time and under conditions sufficient for an antibody-antigen complex to form and determining the level of the complex wherein the level of the complex indicates the level of the ligand bound to the membrane transport protein.

31. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using an assay selected from the group consisting of immunfluorescence, immunohistochemistry, and an immunosorbent assay.

32. The process according to claim 1 or 30 wherein the level of the ligand bound to the membrane transport protein is determined using a fluorescence linked immunosorbent assay.

33. The process according to claim 1 additionally comprising providing the cell expressing the membrane transport protein.

34. The process according to claim 33 wherein providing the cell expressing the membrane protein comprises transforming or transfecting the cell with an expression construct that encodes the membrane protein.

35. The process according to claim 1 additionally comprising fixing the cell.

36. The process according to claim 35 wherein the cell is fixed prior to or at the same time as permeabilizing or disrupting the plasma membrane of the cell.

37. The process according to claim 35 or 36 wherein the cell is fixed with a compound selected from the group consisting of formaldehyde, paraformaldehyde, alcohol, methanol and glutaraldehyde.

38. The process according to claim 35 or 36 wherein the cell is fixed with formaldehyde.

39. The process according to claim 1 additionally comprising inducing translocation of the membrane transport protein to the plasma membrane.

40. The process according to claim 39 wherein inducing translocation of the membrane transport protein to the plasma membrane comprises contacting the cell with an amount of one or more peptides, polypeptides, proteins or compounds sufficient to induce translocation of the membrane transport protein for a time and under conditions sufficient for translocation to occur.

41. The process according to claim 40 wherein the cell is contacted with an amount of sucrose and/or an amount of insulin sufficient to induce translocation.

42. The process according to claim 41 wherein the cell is contacted with sucrose and/or insulin in the presence of serum.

43. The process according to claim 1 additionally comprising inducing resistance to translocation of the membrane transport protein in the cell.

44. The process according to claim 43 wherein the membrane transport is a GLUT protein or a mutant GLUT protein and wherein inducing resistance to translocation of the membrane transport protein in the cell comprises contacting the cell with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient for resistance to insulin induced translocation to occur.

45. The process according to claim 44 wherein the cell is contacted with insulin in the absence of serum.

46. The process according to claim 45 wherein the cell is contacted with insulin for between about 24 hours and about 48 hours.

47. The process of claim 1 comprising:

(a) determining the level of the membrane transport protein at the plasma membrane of a cell using a method comprising:

(i) contacting a cell with a ligand that binds to an extracellular domain of the membrane transport protein for a time and under conditions sufficient for the ligand to bind to the membrane transport protein; and

(ii) determining the level of ligand bound to the membrane transport protein;

(b) determining the level of the membrane transport protein within another cell using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the membrane transport protein within the cell with the ligand for a time and under conditions sufficient for the ligand to bind the membrane transport protein;

(iii) determining the level of ligand bound to the membrane transport protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled membrane transport protein at the plasma membrane relative to the total level of labeled membrane transport protein.

48. The process according to claim 47 wherein the cells are isogenic or from the same cell line.

49. The process according to claim 47 or 48 wherein the cells are cultured under substantially similar conditions.

50. The process according to claim 49 wherein the level of the membrane transport protein at the plasma membrane of the cell and the level of membrane transport protein within the cell are each determined in a plurality of cells.

51. The process according to claim 50 additionally comprising normalizing the determined level of ligand bound to the membrane transport protein with regard to the number of cells in which the level of ligand bound to the membrane transport protein is determined.

52. The process according to claim 51 wherein the number of cells is determined by a method comprising contacting the cells with an antibody or ligand capable of binding to a cell or component thereof for a time and under conditions sufficient for binding of the antibody or ligand to the cell or component thereof and determining the level of antibody bound to the cells, wherein the level of antibody or ligand bound to the cells is indicative of the number of cells.

53. The process according to claim 52 wherein the ligand is wheat germ aggluti

internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(b) determining the level of membrane transport protein within another cell expressing the labeled GLUT4 protein or labeled mutant GLUT4 protein using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(c) comparing the level of ligand detected at (a) (ii) and (b) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

55. A process for determining the level of a labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of a cell that is resistant to insulin induced GLUT4 translocation, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein, said process comprising:

(a) contacting a plurality of cells expressing a labeled GLUT4 protein or a labeled mutant GLUT4 protein with an amount of insulin sufficient to induce resistance to insulin induced translocation for a time and under conditions sufficient to induce resistance to insulin induced GLUT4 translocation in the cell, wherein the cells are contacted with insulin in the absence of serum and wherein

the cells are contacted with insulin for a period of time from about 24 hours to about 48 hours;

(b) determining the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane of a cell at (a) using a method comprising:

(i) contacting the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(ii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(c) determining the level of labeled GLUT4 protein or labeled mutant GLUT4 protein in another cell at (a) but not (b) using a method comprising:

(i) permeabilizing or disrupting the other cell;

(ii) contacting the labeled GLUT4 protein or labeled mutant GLUT4 protein within the cell with a ligand that binds to the label for a time and under conditions sufficient for the ligand to bind to the labeled GLUT4 protein or labeled mutant GLUT4 protein;

(iii) determining the level of ligand bound to the labeled GLUT4 protein or labeled mutant GLUT4 protein; and

(d) comparing the level of ligand detected at (b) (ii) and (c) (iii) to determine the level of the labeled GLUT4 protein or labeled mutant GLUT4 protein at the plasma membrane relative to the total level of labeled GLUT4 protein or labeled mutant GLUT4 protein.

56. A process for determining the level of recycling of a membrane transport protein in a cell or a change in the level of recycling of a cell comprising:

(a) determining the level of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of another cell using the process according to any one of claims 1 to 54, wherein the other cell is cultured for a longer period of time than the cell at (a); and

(c) comparing the level of the membrane transport protein translocated to the plasma membrane at (a) and (b) to thereby determine the level of recycling of the membrane transport protein in the cell, wherein a change in the level of the

membrane transport protein translocated to the plasma membrane indicates a change in the level of recycling of a membrane transport protein.

57. A process for determining a mutation in a nucleic acid encoding a mutant membrane transport protein that is capable of modulating translocation of said membrane transport protein, said method comprising:

(i) determining the level of the mutant membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54; and

(ii) determining the level of the wild-type form of the membrane transport protein translocated to the plasma membrane of a cell using the process according to any one of claims 1 to 54,

wherein an enhanced or suppressed level of translocation of the membrane transport protein at (a) compared to (b) indicates that the nucleic acid comprises a mutation that is capable of modulating the level of level of translocation of the membrane transport protein to the plasma membrane.

58. A process for determining an agent that modulates translocation of a membrane transport protein to the plasma membrane of a cell, said process comprising:

(a) determining the level of a membrane transport protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to any one of claims 1 to 54;

(b) determining the level of the membrane transport protein translocated to the plasma membrane of a cell in the presence of the candidate agent by performing the process according to any one of claims 1 to 54, wherein a difference in the level of the membrane transport protein translocated to the plasma membrane of a cell at (a) compared to (b) indicates that the candidate agent modulates translocation of the membrane transport protein.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(e) optionally, providing, the candidate agent.

59. A process for determining a candidate compound for the treatment of insulin resistance comprising:

(a) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a

candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(b) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

(c) optionally, determining the structure of the candidate agent;

(d) optionally, providing the name or structure of the candidate agent; and

(e) optionally, providing, the candidate agent.

60. The process of claim 59 wherein the insulin resistance is associated with diabetes.

61. The process according to claim 60 wherein the diabetes is type II diabetes.

62. A process for manufacturing a medicament for the treatment of insulin resistance comprising:

(a) determining a candidate compound for the treatment of insulin resistance using a process comprising:

(i) determining the level of the labeled GLUT4 protein or the labeled mutant GLUT4 protein translocated to the plasma membrane of a cell in the absence of a candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein; and

(ii) determining the level of the labeled GLUT4 protein or a labeled mutant GLUT4 protein translocated to the plasma membrane of another cell in the presence of the candidate agent by performing the process according to claim 55, wherein said labeled mutant GLUT4 protein has a reduced rate of recycling or transporter internalization compared to a wild-type form of the membrane transport protein and wherein a candidate agent that enhances the

level of translocation of the labeled GLUT4 protein or a labeled mutant GLUT4 protein is a candidate agent for the treatment of insulin resistance.

(b) optionally, isolating the candidate agent;

(c) optionally, providing the name or structure of the candidate agent;

(d) optionally, providing the candidate agent; and

(e) using the candidate agent in the manufacture of a medicament for the treatment of insulin resistance.

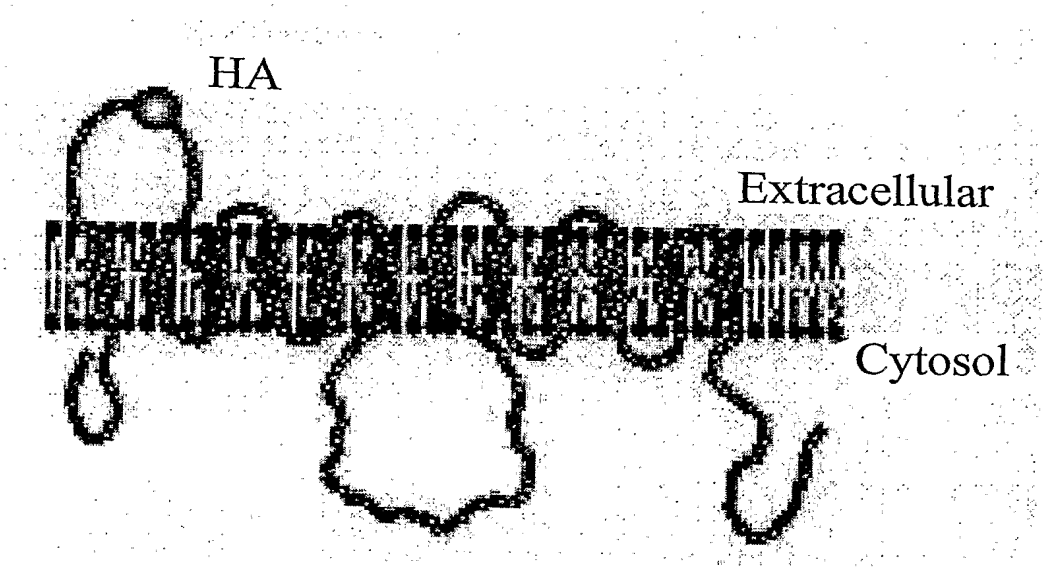

Figure 1a

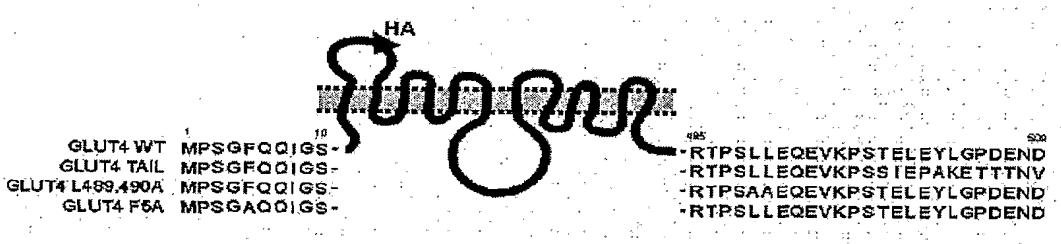
HA
GLUT4 WT MPSGFQQIGS-
GLUT4 TAIL MPSGFQQIGS-
GLUT4 L489,490A MPSGFQQIGS-
GLUT4 F5A MPSGAQQIGS-
-RTPSLLEQEVKPSTELEYLGPDEND
-RTPSLLEQEVKPSSIEPAKETTTNV
-RTPSAAEQEVKPSTELEYLGPDEND
-RTPSLLEQEVKPSTELEYLGPDEND

Figure 1b

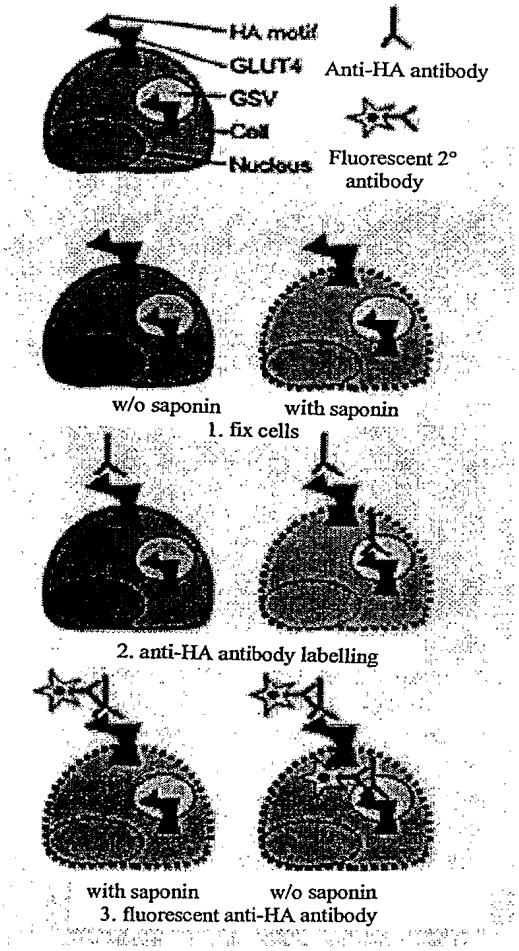
HA motif
GLUT4
GSV
Cell
Nucleus
Anti-HA antibody
Fluorescent 2° antibody
w/o saponin
with saponin
1. fix cells
2. anti-HA antibody labelling
with saponin
w/o saponin
3. fluorescent anti-HA antibody

Figure 1c

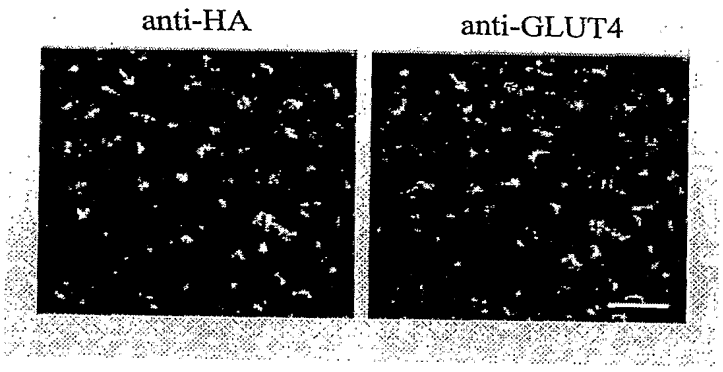

Figure 1d

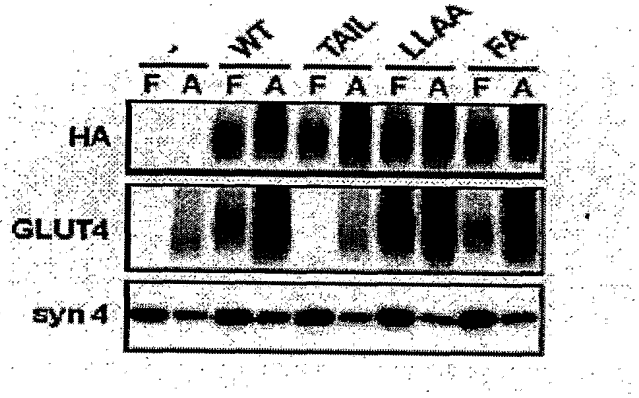

Figure 1e

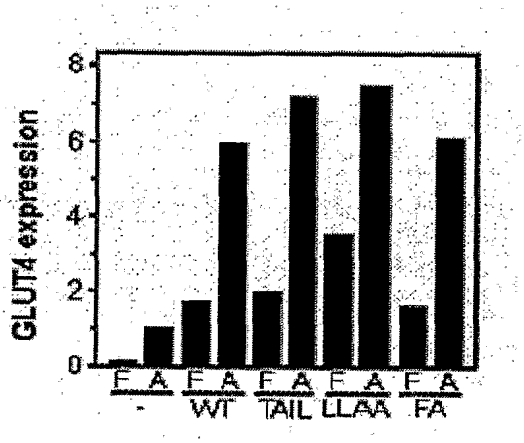

Figure 1f

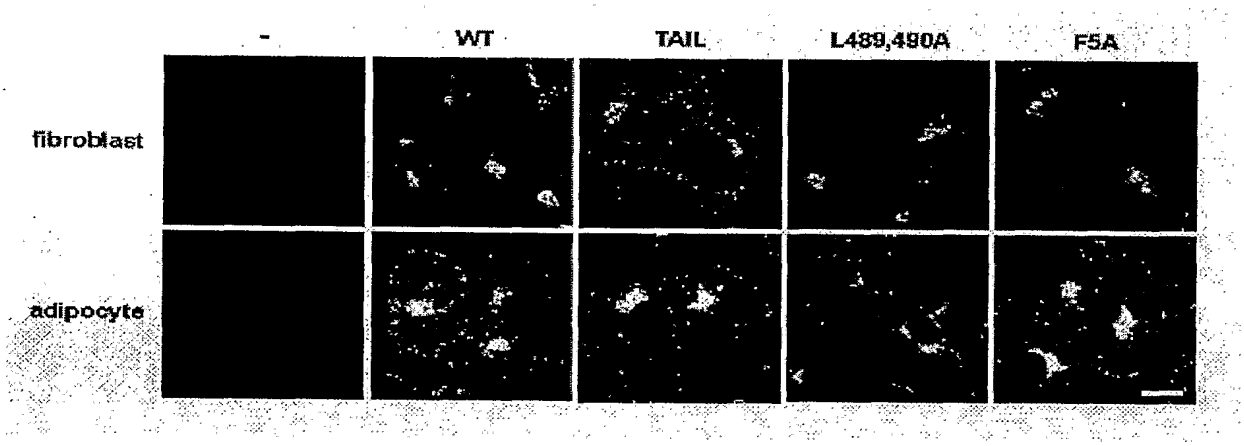

Figure 1g

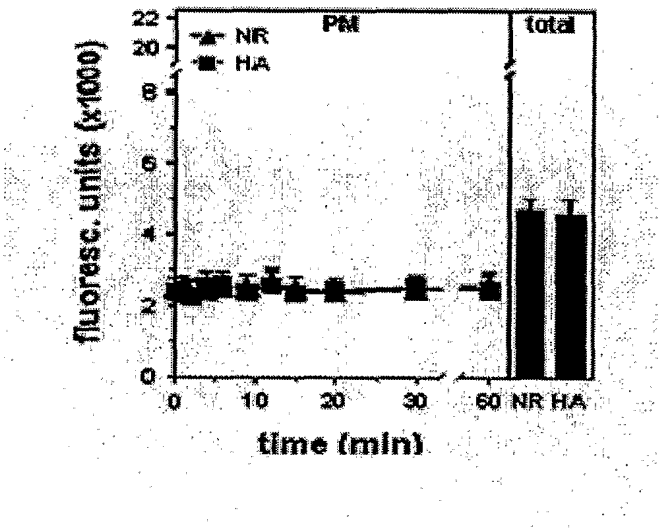
PM
total
NR
HA
fluoresc. units (x1000)
22
20
8
6
4
2
0
0
10
20
30
60
NR
HA
time (min)

Figure 2a

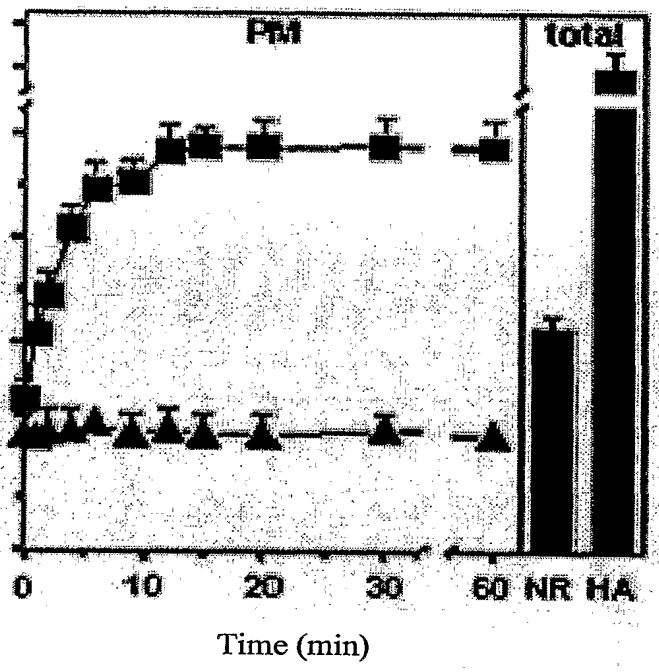

Figure 2b

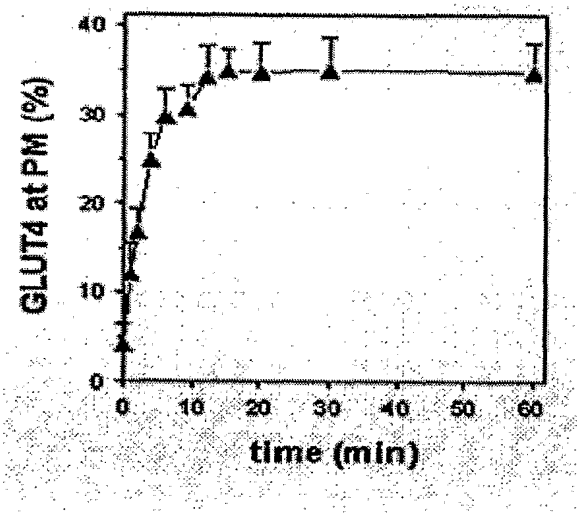

Figure 2c

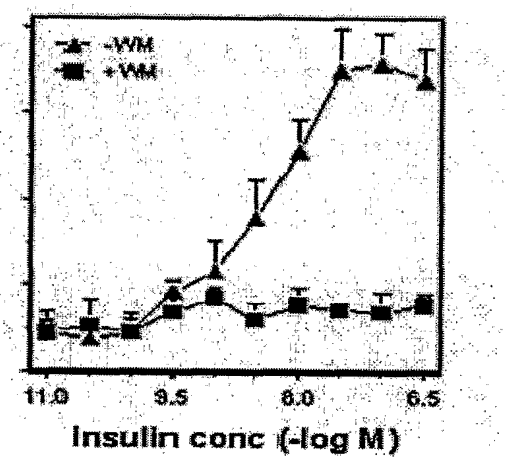

Figure 2d

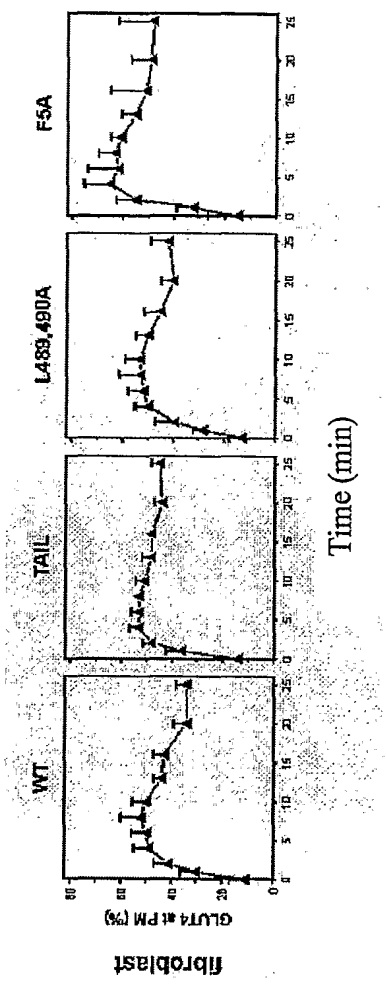

Figure 3a

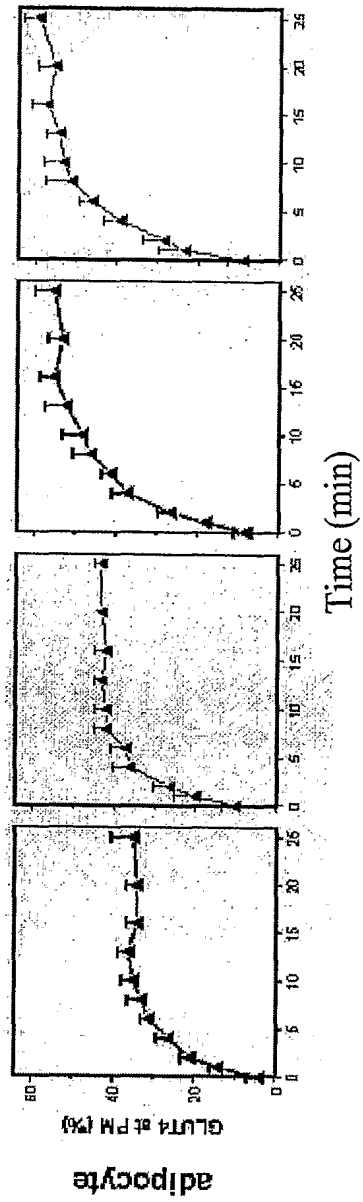

Figure 3b

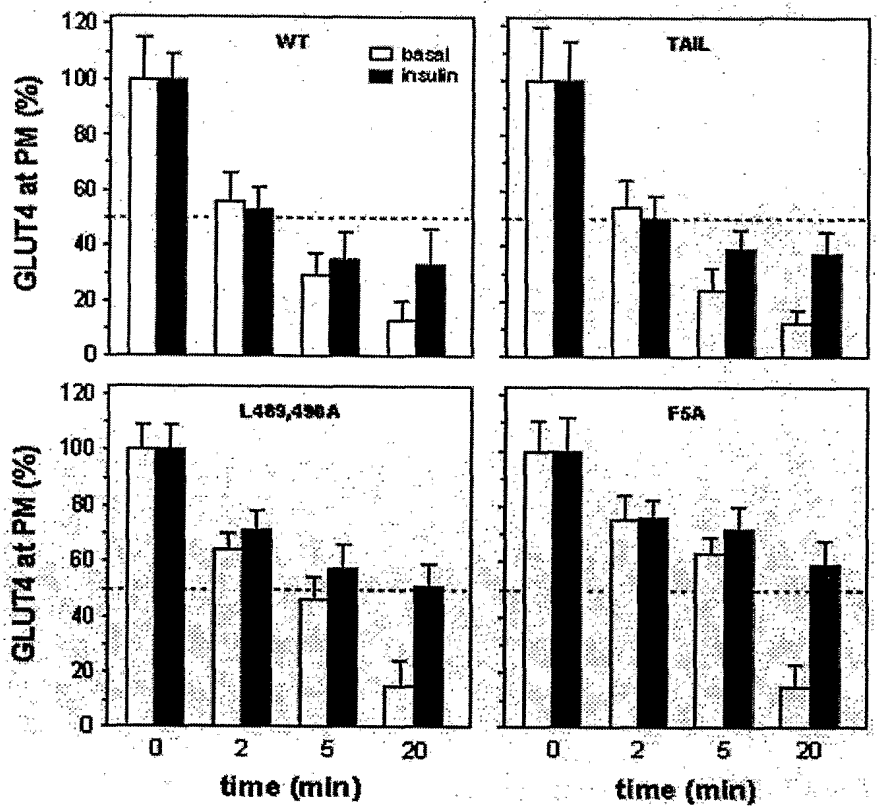
WT
basal
insulin
TAIL
L469,498A
F5A
GLUT4 at PM (%)
GLUT4 at PM (%)
0
20
40
60
80
100
120
0
2
5
20
time (min)
time (min)

Figure 4

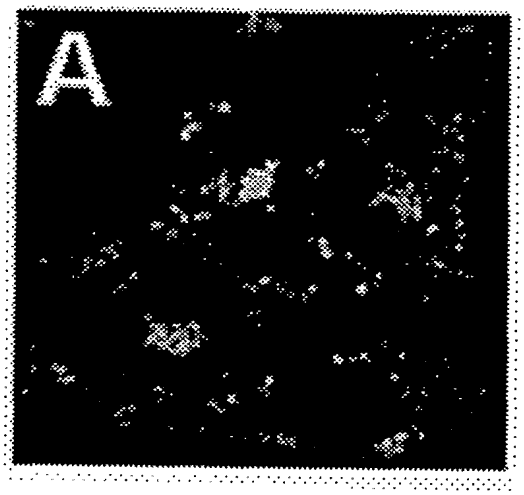

Figure 5a

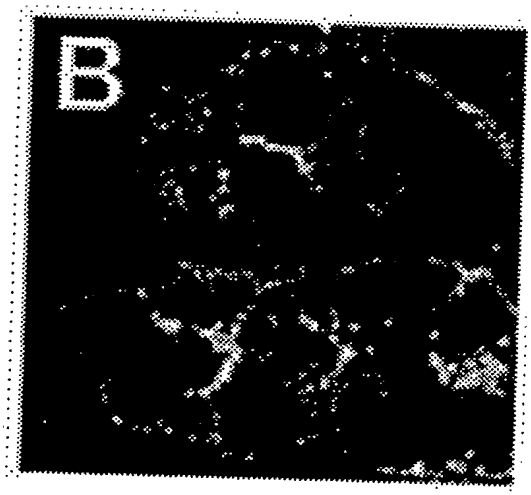

Figure 5b

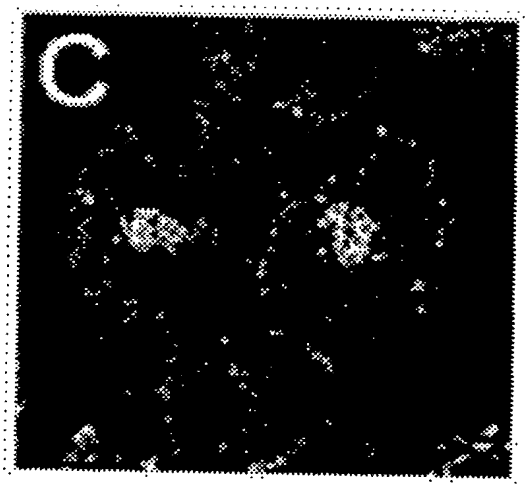

Figure 5c

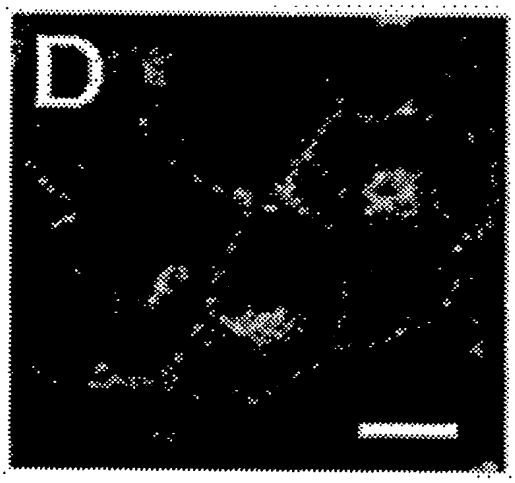

Figure 5d

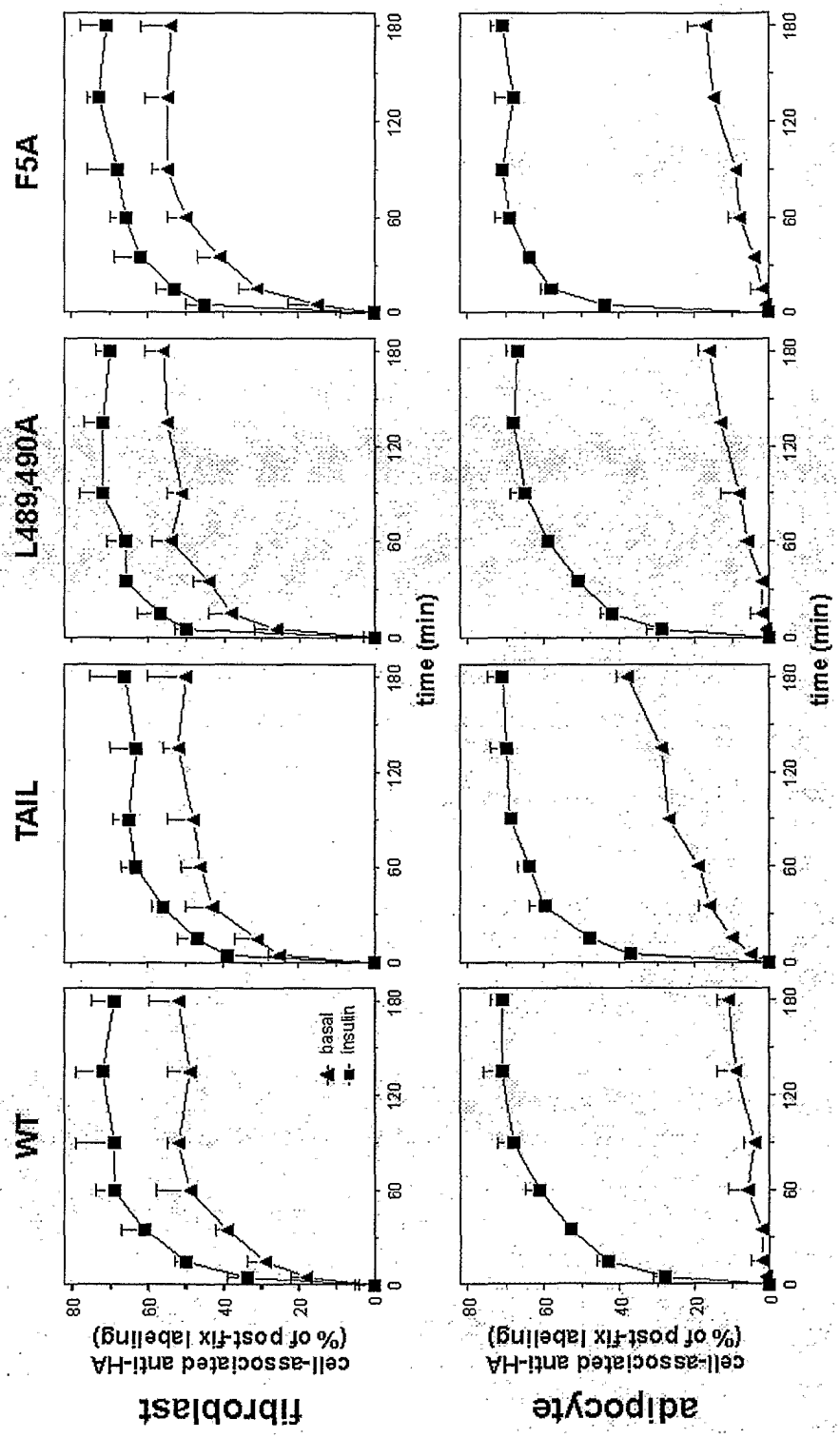
WT
TAIL
L489,490A
F5A
basal
insulin
fibroblast
adipocyte
cell-associated anti-HA
(% of post-fix labeling)
time (min)
0
20
40
60
80
0
60
120
180

Figure 5e

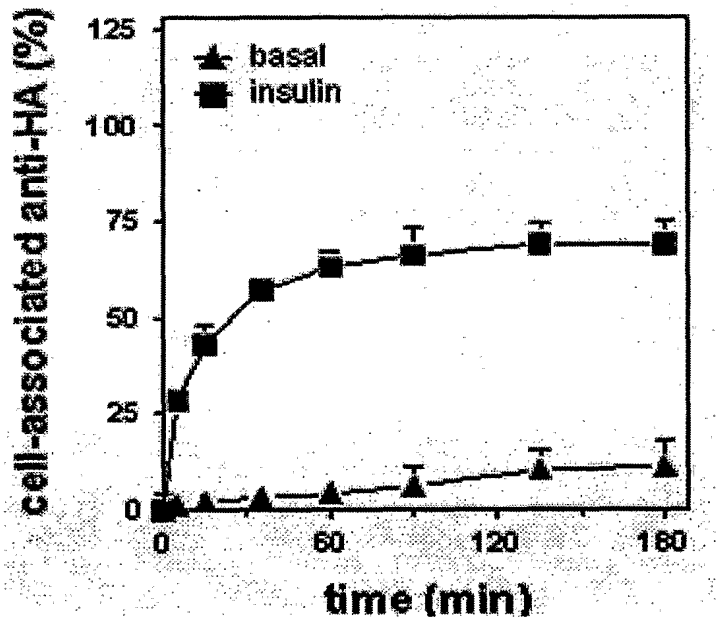
cell-associated anti-HA (%)
125
100
75
50
25
0
basal
insulin
0
60
120
180
time (min)

Figure 6a

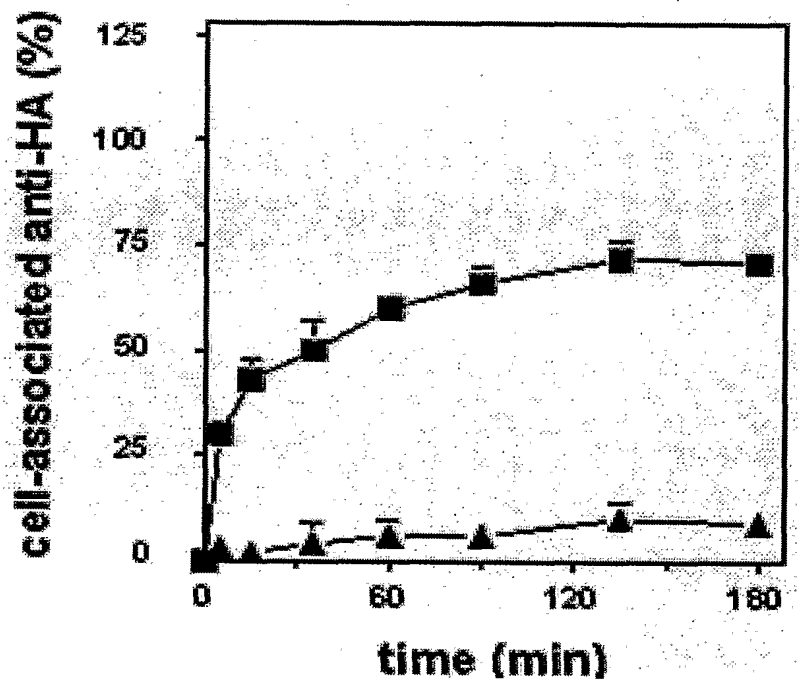

Figure 6b

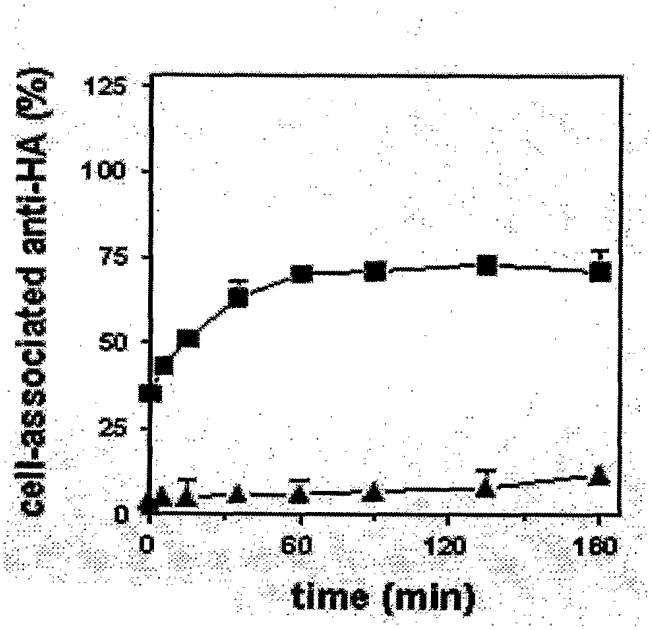

Figure 6c

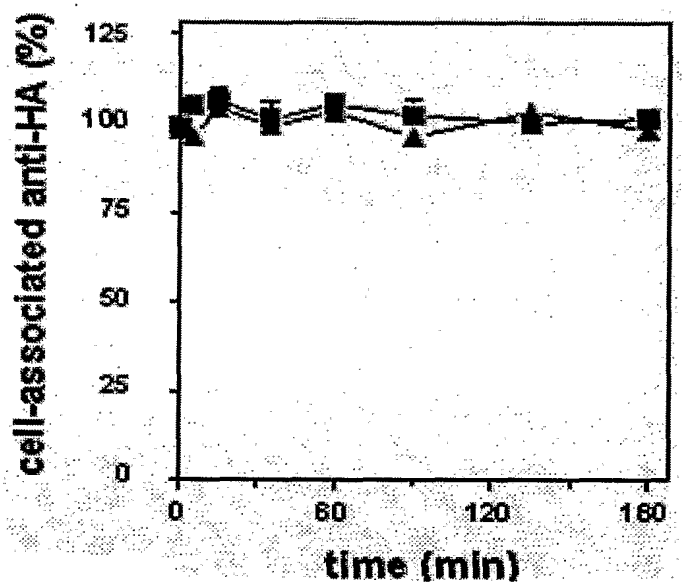

Figure 6d

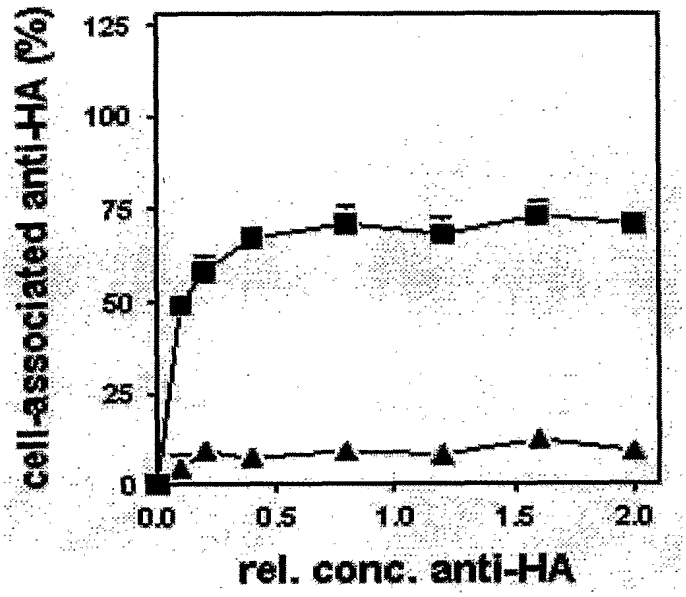
cell-associated anti-HA (%)
125
100
75
50
25
0
0.0
0.5
1.0
1.5
2.0
rel. conc. anti-HA

Figure 6e

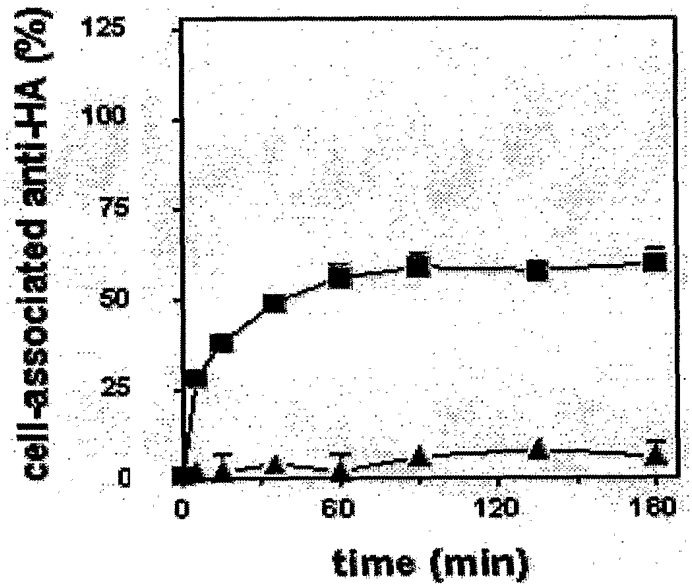

Figure 6f

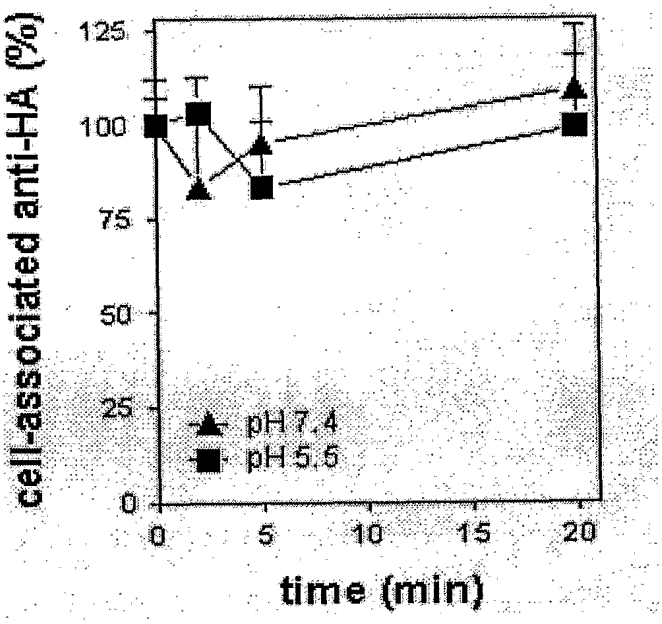
cell-associated anti-HA (%)
125
100
75
50
25
0
0
5
10
15
20
time (min)
pH 7.4
pH 5.5

Figure 6g

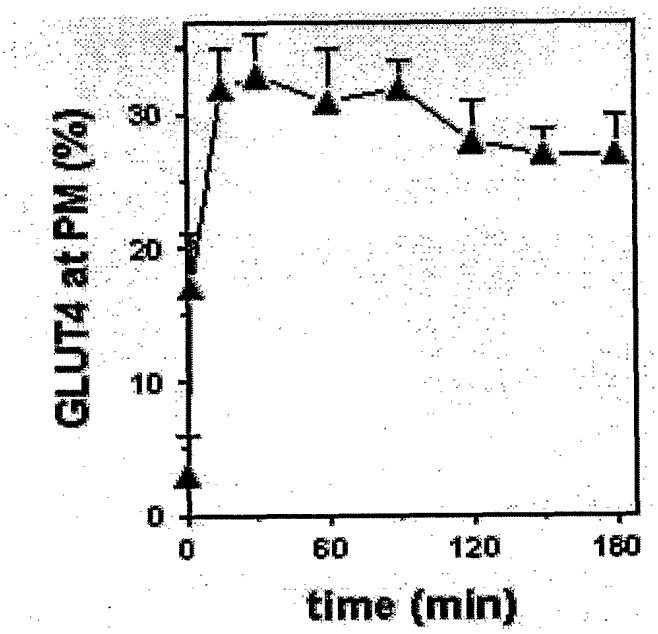

Figure 6h

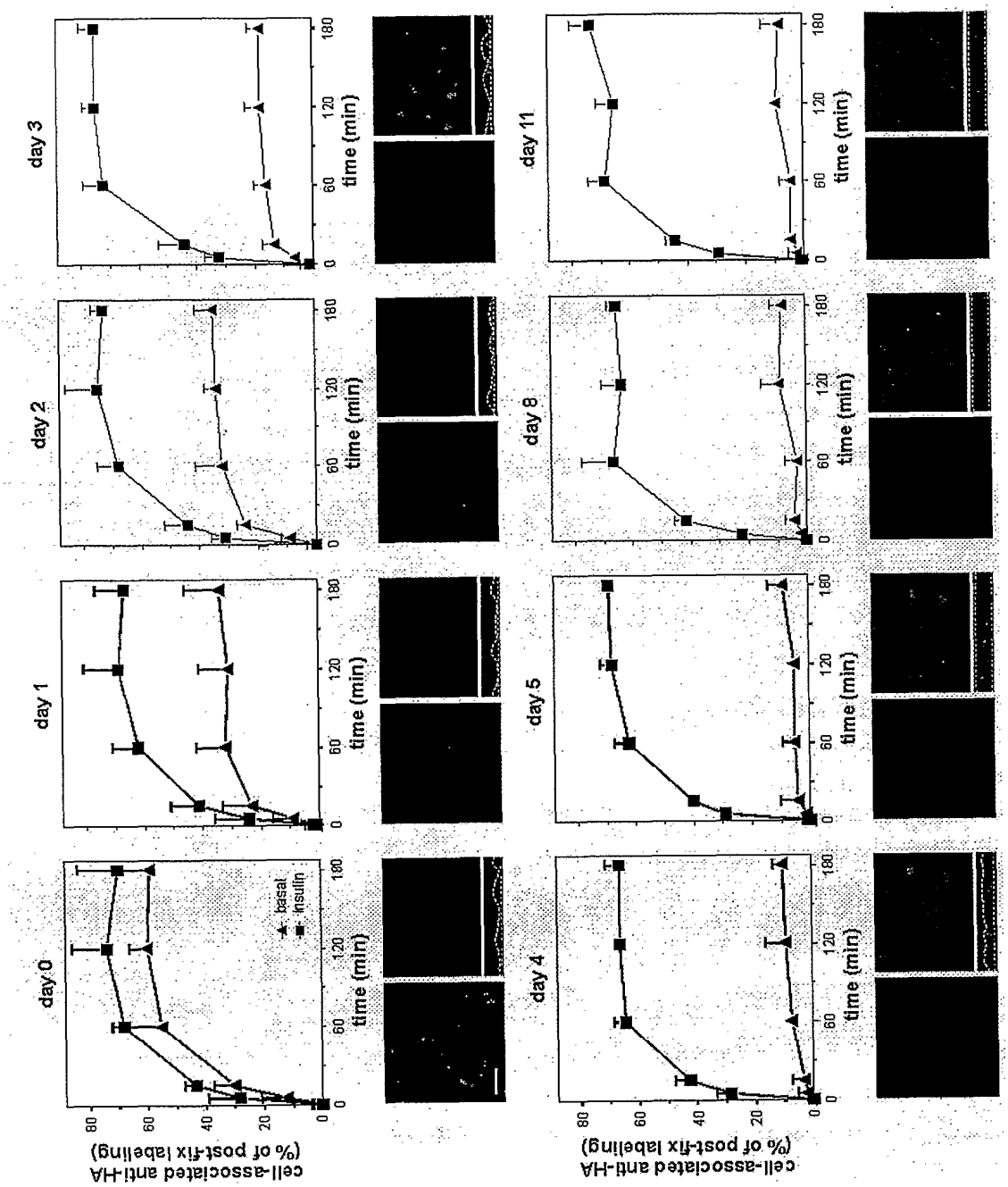
day 0
day 1
day 2
day 3
day 4
day 5
day 8
day 11
basal
insulin
time (min)
cell-associated anti-HA
(% of post-fix labeling)

Figure 7

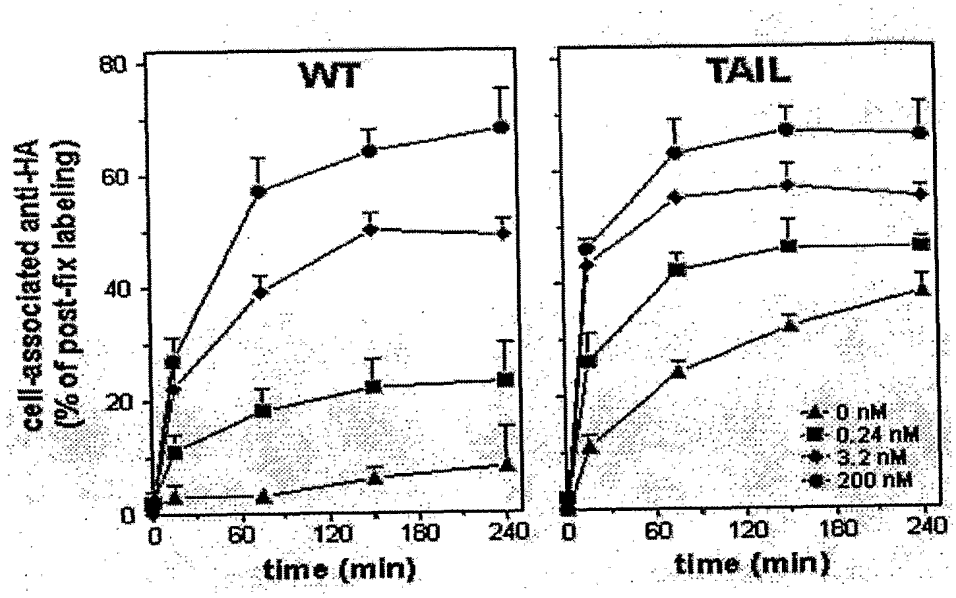
WT
TAIL
cell-associated anti-HA
(% of post-fix labeling)
80
60
40
20
0
0
60
120
180
240
time (min)
0 nM
0.24 nM
3.2 nM
200 nM

Figure 8a

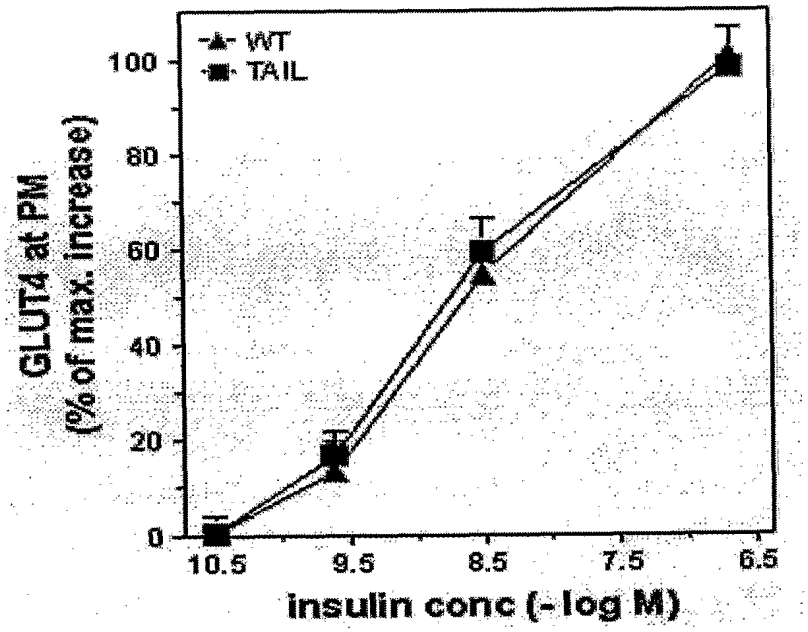
WT
TAIL
GLUT4 at PM
(% of max. increase)
100
80
60
40
20
0
10.5
9.5
8.5
7.5
6.5
insulin conc (- log M)

Figure 8b

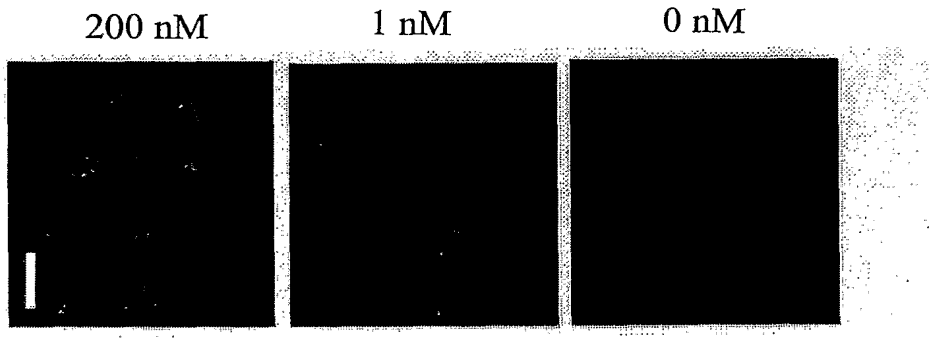

Figure 8c

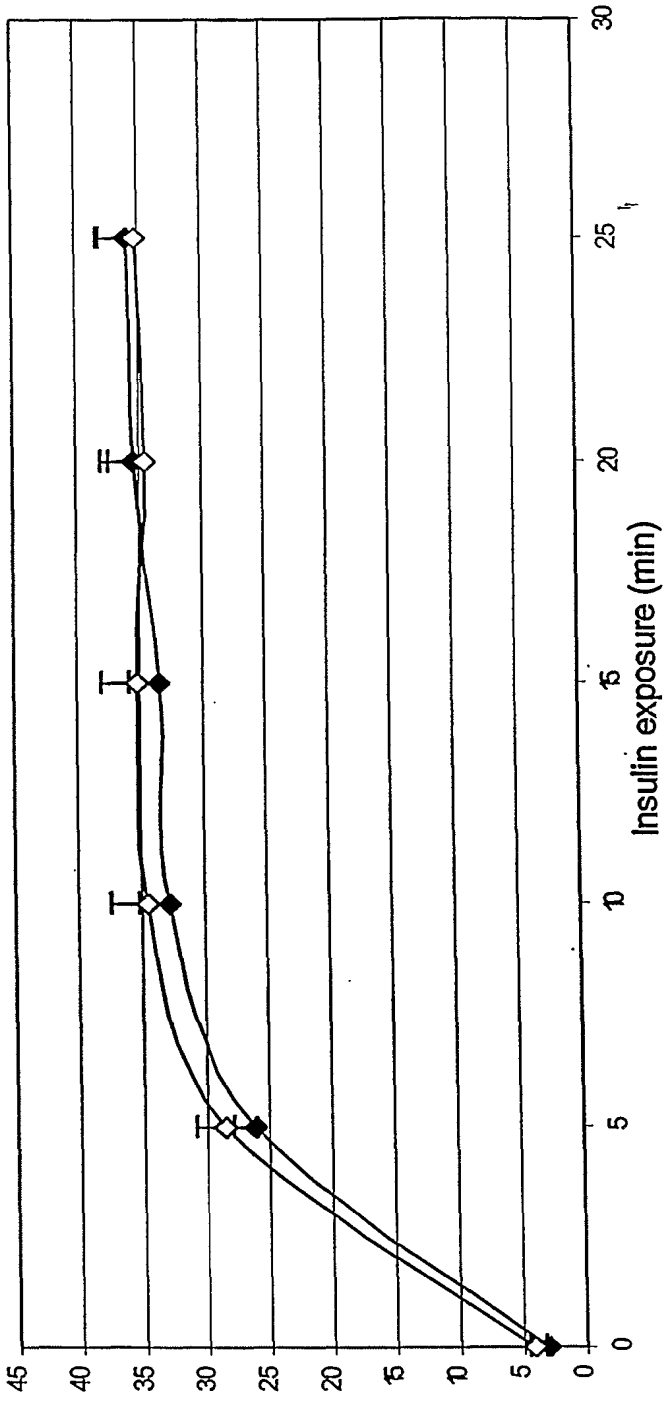

Figure 9

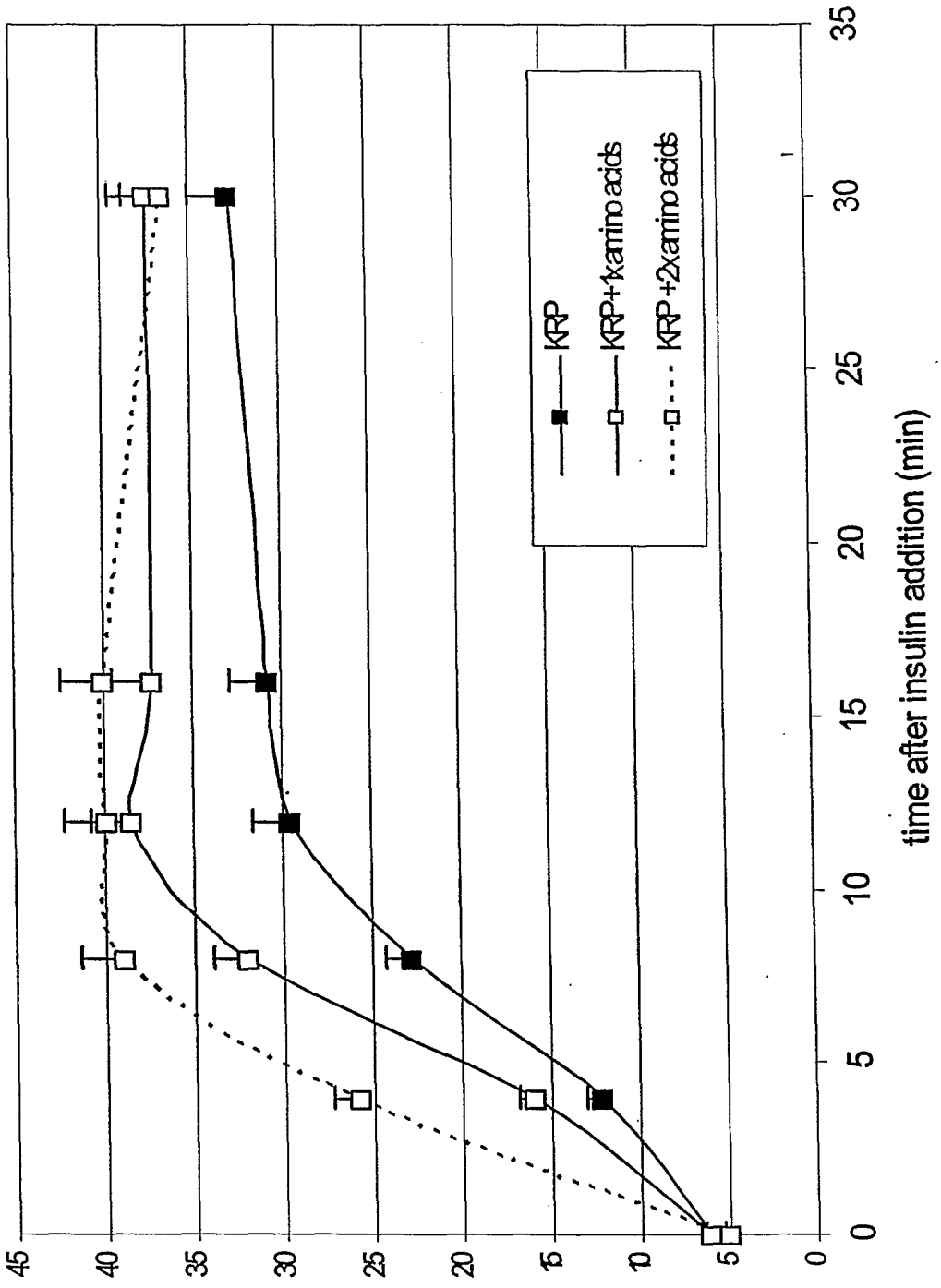
KRP
KRP+1xamino acids
KRP +2xamino acids
time after insulin addition (min)
0
5
10
15
20
25
30
35
45
40
35
30
25
20
15
10
5
0

Figure 10

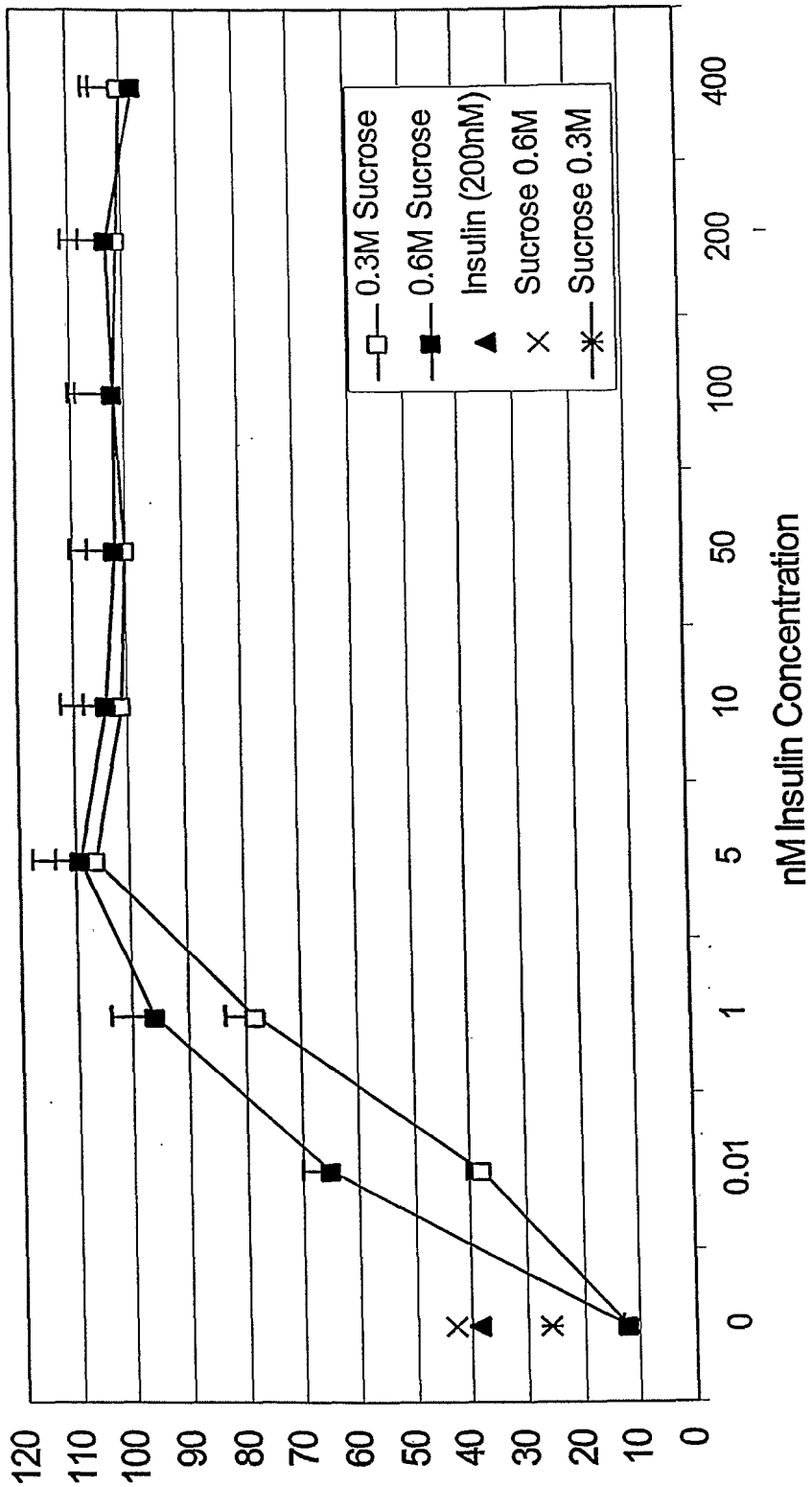
0.3M Sucrose
0.6M Sucrose
Insulin (200nM)
Sucrose 0.6M
Sucrose 0.3M
nM Insulin Concentration
0
0.01
1
5
10
50
100
200
400
0
10
20
30
40
50
60
70
80
90
100
110
120

Figure 11

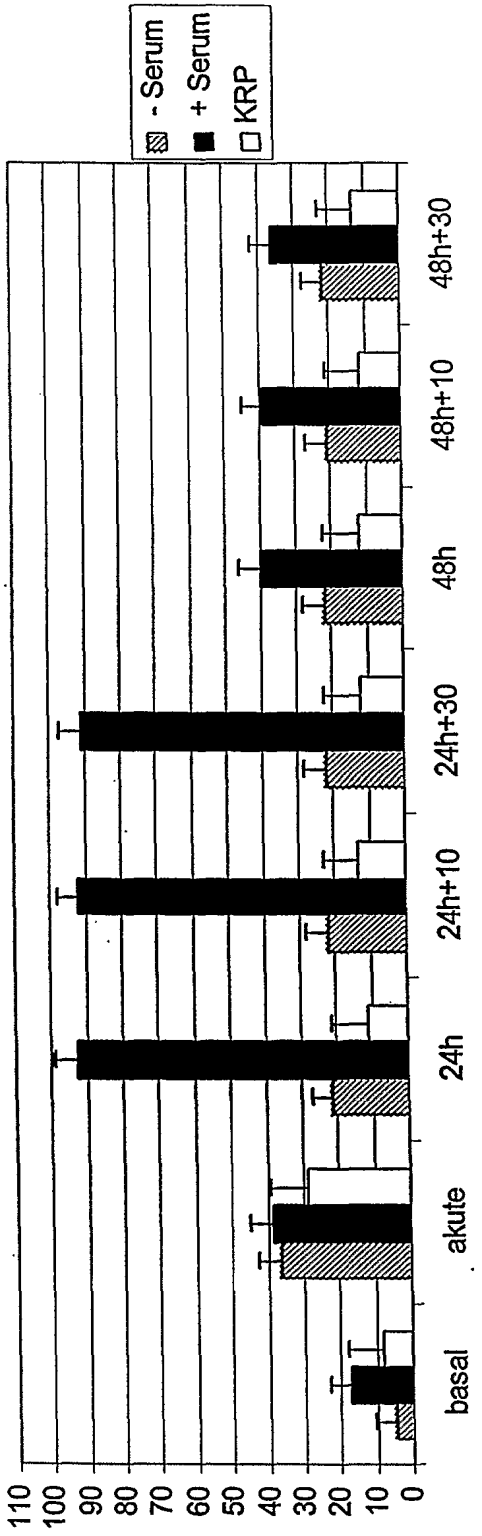
- Serum
+ Serum
KRP
48h+30
48h+10
48h
24h+30
24h+10
24h
akute
basal
110
100
90
80
70
60
50
40
30
20
10
0

Figure 12A

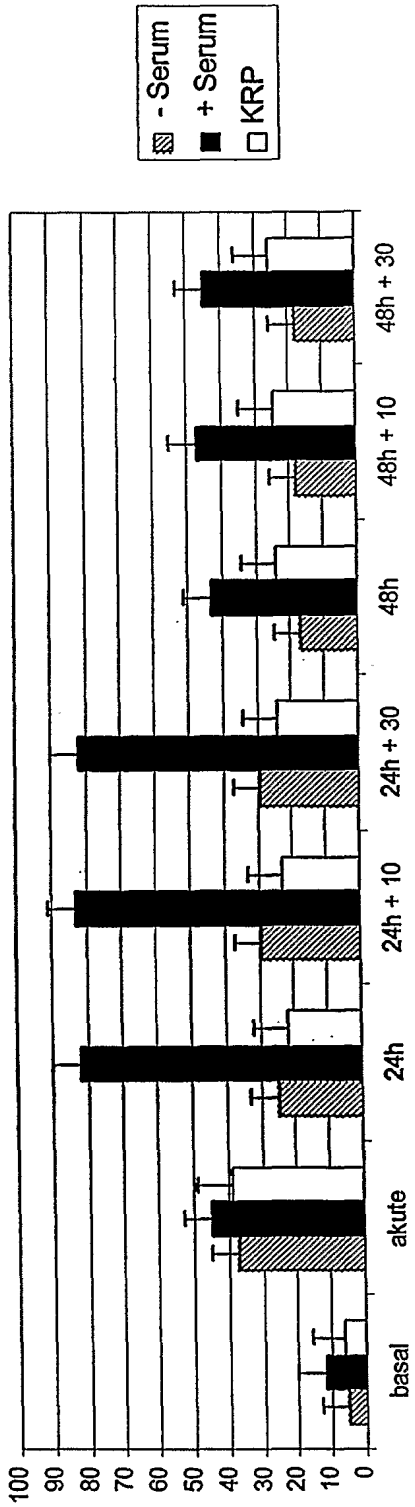
- Serum
+ Serum
KRP
48h + 30
48h + 10
48h
24h + 30
24h + 10
24h
akute
basal
100
90
80
70
60
50
40
30
20
10
0

Figure 12B

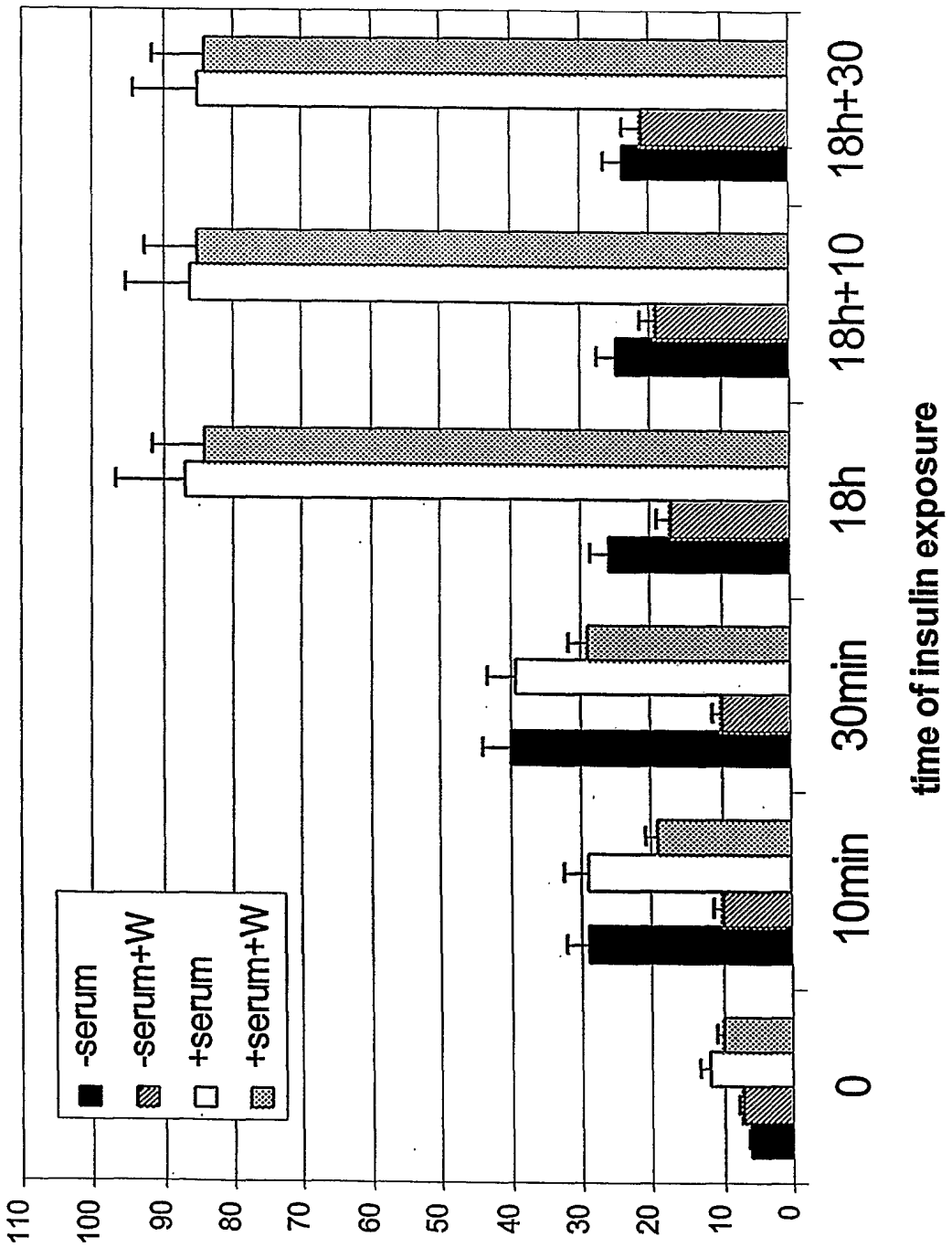
-serum
-serum+W
+serum
+serum+W
110
100
90
80
70
60
50
40
30
20
10
0
0
10min
30min
18h
18h+10
18h+30
time of insulin exposure

Figure 13

SEQUENCE LISTING

<110> Garvan Institute of Medical Research

<120> Novel Translocation Assay

<130> 502551/PXM

<150> AU2003904237
<151> 2003-08-08

<160> 64

<170> PatentIn version 3.1

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<212> DNA
<213> GLUT4

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tctgcgctcc aggccggagt cagagactcc aggatcggtt ctttcatctt cgccgccccct 120

gcgcgtccag ctcttctaag acgag atg ccg tcg ggc ttc caa cag ata ggc 172
Met Pro Ser Gly Phe Gln Gln Ile Gly
1 5

tcc gaa gat ggg gaa ccc cct cag cag cga gtg act ggg acc ctg gtc 220
Ser Glu Asp Gly Glu Pro Pro Gln Gln Arg Val Thr Gly Thr Leu Val
10 15 20 25

ctt gct gtg ttc tct gcg gtg ctt ggc tcc ctg cag ttt ggg tac aac 268
Leu Ala Val Phe Ser Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn
30 35 40

att ggg gtc atc aat gcc cct cag aag gtg att gaa cag agc tac aat 316
Ile Gly Val Ile Asn Ala Pro Gln Lys Val Ile Glu Gln Ser Tyr Asn
45 50 55

gag acg tgg ctg ggg agg cag ggg cct gag gga ccc agc tcc atc cct 364
Glu Thr Trp Leu Gly Arg Gln Gly Pro Glu Gly Pro Ser Ser Ile Pro
60 65 70

cca ggc acc ctc acc acc ctc tgg gcc ctc tcc gtg gcc atc ttt tcc 412
Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser
75 80 85

gtg ggc ggc atg att tcc tcc ttc ctc att ggt atc atc tct cag tgg 460
Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp
90 95 100 105

ctt gga agg aaa agg gcc atg ctg gtc aac aat gtc ctg gcg gtg ctg 508
Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu
110 115 120

ggg ggc agc ctc atg ggc ctg gcc aac gct gct gcc tcc tat gaa atg 556

```
Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met
            125                 130                 135

ctc atc ctt gga cga ttc ctc att ggc gcc tac tca ggg ctg aca tca      604
Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser
        140                 145                 150

ggg ctg gtg ccc atg tac gtg ggg gag att gct ccc act cac ctg cgg      652
Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg
    155                 160                 165

ggc gcc ctg ggg acg ctc aac caa ctg gcc att gtt atc ggc att ctg      700
Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu
170                 175                 180                 185

atc gcc cag gtg ctg ggc ttg gag tcc ctc ctg ggc act gcc agc ctg      748
Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu
                190                 195                 200

tgg cca ctg ctc ctg ggc ctc aca gtg cta cct gcc ctc ctg cag ctg      796
Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu
            205                 210                 215

gtc ctg ctg ccc ttc tgt ccc gag agc ccc cgc tac ctc tac atc atc      844
Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile
        220                 225                 230

cag aat ctc gag ggg cct gcc aga aag agt ctg aag cgc ctg aca ggc      892
Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly
    235                 240                 245

tgg gcc gat gtt tct gga gtg ctg gct gag ctg aag gat gag aag cgg      940
Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg
250                 255                 260                 265

aag ctg gag cgt gag cgg cca ctg tcc ctg ctc cag ctc ctg ggc agc      988
Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser
                270                 275                 280

cgt acc cac cgg cag ccc ctg atc att gcg gtc gtg ctg cag ctg agc     1036
Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser
            285                 290                 295

cag cag ctc tct ggc atc aat gct gtt ttc tat tat tcg acc agc atc     1084
Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile
        300                 305                 310

ttc gag aca gca ggg gta ggc cag cct gcc tat gcc acc ata gga gct     1132
Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala
    315                 320                 325

ggt gtg gtc aac aca gtc ttc acc ttg gtc tcg gtg ttg ttg gtg gag     1180
Gly Val Val Asn Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu
330                 335                 340                 345

cgg gcg ggg cgc cgg acg ctc cat ctc ctg ggc ctg gcg ggc atg tgt     1228
Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys
                350                 355                 360

ggc tgt gcc atc ctg atg act gtg gct ctg ctc ctg ctg gag cga gtt     1276
Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val
            365                 370                 375
```

```
cca gcc atg agc tac gtc tcc att gtg gcc atc ttt ggc ttc gtg gca     1324
Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala
        380                 385                 390

ttt ttt gag att ggc cct ggc ccc att cct tgg ttc atc gtg gcc gag     1372
Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu
    395                 400                 405

ctc ttc agc cag gga ccc cgc ccg gca gcc atg gct gtg gct ggt ttc     1420
Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe
410                 415                 420                 425

tcc aac tgg acg agc aac ttc atc att ggc atg ggt ttc cag tat gtt     1468
Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val
                430                 435                 440

gcg gag gct atg ggg ccc tac gtc ttc ctt cta ttt gcg gtc ctc ctg     1516
Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu
            445                 450                 455

ctg ggc ttc ttc atc ttc acc ttc tta aga gta cct gaa act cga ggc     1564
Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly
        460                 465                 470

cgg acg ttt gac cag atc tca gct gcc ttc cac cgg aca ccc tct ctt     1612
Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu
    475                 480                 485

tta gag cag gag gtg aaa ccc agc aca gaa ctt gag tat tta ggg cca     1660
Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro
490                 495                 500                 505

gat gag aac gac tgaggggcca ggcaggggtg ggagagccag ctctctctac        1712
Asp Glu Asn Asp


ccggcccaga gacccttcc tttcctctgc agcactttaa ccctctcttc cctattattt   1772

ccgggtggaa aagaatccct gcagcctggt agaattggga agctgggggga agggtggtct  1832

gagcaccccc tcattcccct cgtgtgactc tcttggatta tttatgtgtt gtggtttggc   1892

cgtggccatc agggtgggcc actctcccct ccctcttcct tcccccatcc cctttcctcc   1952

ccaccttccc cagactcagc tccagaatac cttcttcgct gctagagaag ggggattgga   2012

gggaagacag gtctagactt tctcagtggg acaaaccaga gcagagagca ggacaggaga   2072

caagaaatcc agtttcccac caccttggac tcctcccaca atctgggact ttcact       2128


<210>  2
<211>  509
<212>  PRT
<213>  GLUT4

<400>  2

Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
```

```
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu
65                  70                  75                  80

Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser
                85                  90                  95

Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met
            100                 105                 110

Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu
        115                 120                 125

Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu
    130                 135                 140

Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val
145                 150                 155                 160

Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn
                165                 170                 175

Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu
            180                 185                 190

Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu
        195                 200                 205

Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro
    210                 215                 220

Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala
225                 230                 235                 240

Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val
                245                 250                 255

Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro
            260                 265                 270
```

```
Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu
        275                 280                 285

Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn
    290                 295                 300

Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly
305                 310                 315                 320

Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe
                325                 330                 335

Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu
            340                 345                 350

His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr
        355                 360                 365

Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser
    370                 375                 380

Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly
385                 390                 395                 400

Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg
                405                 410                 415

Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe
            420                 425                 430

Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr
        435                 440                 445

Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr
    450                 455                 460

Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser
465                 470                 475                 480

Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro
                485                 490                 495

Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
            500                 505
```

<210> 3
<211> 1566
<212> DNA

<213> HA tagged GLUT4

<220>
<221> CDS
<222> (1)..(1566)
<223>

<400> 3

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atg ccg tcg ggc ttc caa cag ata ggc tcc gaa gat ggg gaa ccc cct       48
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                  10                  15

cag cag cga gtg act ggg acc ctg gtc ctt gct gtg ttc tct gcg gtg       96
Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

ctt ggc tcc ctg cag ttt ggg tac aac att ggg gtc atc aat gcc cct      144
Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

cag aag gtg att gaa cag agc tac aat gag acg tgg ctg ggg agg cag      192
Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

ggg cct gag atc gat tat cct tat gat gtt cct gat tat gct gag gga      240
Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
65                  70                  75                  80

ccc agc tcc atc cct cca ggc acc ctc acc acc ctc tgg gcc ctc tcc      288
Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
                85                  90                  95

gtg gcc atc ttt tcc gtg ggc ggc atg att tcc tcc ttc ctc att ggt      336
Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
            100                 105                 110

atc atc tct cag tgg ctt gga agg aaa agg gcc atg ctg gtc aac aat      384
Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
        115                 120                 125

gtc ctg gcg gtg ctg ggg ggc agc ctc atg ggc ctg gcc aac gct gct      432
Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
    130                 135                 140

gcc tcc tat gaa atg ctc atc ctt gga cga ttc ctc att ggc gcc tac      480
Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
145                 150                 155                 160

tca ggg ctg aca tca ggg ctg gtg ccc atg tac gtg ggg gag att gct      528
Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
                165                 170                 175

ccc act cac ctg cgg ggc gcc ctg ggg acg ctc aac caa ctg gcc att      576
Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
            180                 185                 190

gtt atc ggc att ctg atc gcc cag gtg ctg ggc ttg gag tcc ctc ctg      624
Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
        195                 200                 205

ggc act gcc agc ctg tgg cca ctg ctc ctg ggc ctc aca gtg cta cct      672
Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
    210                 215                 220
```

```
gcc ctc ctg cag ctg gtc ctg ctg ccc ttc tgt ccc gag agc ccc cgc      720
Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
225                 230                 235                 240

tac ctc tac atc atc cag aat ctc gag ggg cct gcc aga aag agt ctg      768
Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
                245                 250                 255

aag cgc ctg aca ggc tgg gcc gat gtt tct gga gtg ctg gct gag ctg      816
Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu
            260                 265                 270

aag gat gag aag cgg aag ctg gag cgt gag cgg cca ctg tcc ctg ctc      864
Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu
        275                 280                 285

cag ctc ctg ggc agc cgt acc cac cgg cag ccc ctg atc att gcg gtc      912
Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val
    290                 295                 300

gtg ctg cag ctg agc cag cag ctc tct ggc atc aat gct gtt ttc tat      960
Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
305                 310                 315                 320

tat tcg acc agc atc ttc gag aca gca ggg gta ggc cag cct gcc tat     1008
Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr
                325                 330                 335

gcc acc ata gga gct ggt gtg gtc aac aca gtc ttc acc ttg gtc tcg     1056
Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser
            340                 345                 350

gtg ttg ttg gtg gag cgg gcg ggg cgc cgg acg ctc cat ctc ctg ggc     1104
Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly
        355                 360                 365

ctg gcg ggc atg tgt ggc tgt gcc atc ctg atg act gtg gct ctg ctc     1152
Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu
    370                 375                 380

ctg ctg gag cga gtt cca gcc atg agc tac gtc tcc att gtg gcc atc     1200
Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile
385                 390                 395                 400

ttt ggc ttc gtg gca ttt ttt gag att ggc cct ggc ccc att cct tgg     1248
Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
                405                 410                 415

ttc atc gtg gcc gag ctc ttc agc cag gga ccc cgc ccg gca gcc atg     1296
Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
            420                 425                 430

gct gtg gct ggt ttc tcc aac tgg acg agc aac ttc atc att ggc atg     1344
Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
        435                 440                 445

ggt ttc cag tat gtt gcg gag gct atg ggg ccc tac gtc ttc ctt cta     1392
Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
    450                 455                 460

ttt gcg gtc ctc ctg ctg ggc ttc ttc atc ttc acc ttc tta aga gta     1440
Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
```

```
465                 470                 475                 480

cct gaa act cga ggc cgg acg ttt gac cag atc tca gct gcc ttc cac     1488
Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
                485                 490                 495

cgg aca ccc tct ctt tta gag cag gag gtg aaa ccc agc aca gaa ctt     1536
Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
            500                 505                 510

gag tat tta ggg cca gat gag aac gac tga                            1566
Glu Tyr Leu Gly Pro Asp Glu Asn Asp
        515                 520
```

<210> 4
<211> 521
<212> PRT
<213> HA tagged GLUT4

<400> 4

```
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
65                  70                  75                  80

Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
                85                  90                  95

Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
            100                 105                 110

Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
        115                 120                 125

Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
    130                 135                 140

Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
145                 150                 155                 160

Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
```

```
                165                 170                 175

Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
            180                 185                 190

Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
        195                 200                 205

Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
    210                 215                 220

Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
225                 230                 235                 240

Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
                245                 250                 255

Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu
            260                 265                 270

Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu
        275                 280                 285

Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val
    290                 295                 300

Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
305                 310                 315                 320

Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr
                325                 330                 335

Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser
            340                 345                 350

Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly
        355                 360                 365

Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu
    370                 375                 380

Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile
385                 390                 395                 400

Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
                405                 410                 415
```

```
Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
            420                 425                 430

Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
        435                 440                 445

Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
    450                 455                 460

Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
465                 470                 475                 480

Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
                485                 490                 495

Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
            500                 505                 510

Glu Tyr Leu Gly Pro Asp Glu Asn Asp
        515                 520
```

<210> 5
<211> 512
<212> PRT
<213> GLUT4 TAIL mutant

<400> 5

```
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
65                  70                  75                  80

Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
                85                  90                  95

Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
            100                 105                 110
```

```
Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
        115                 120                 125

Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
    130                 135                 140

Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
145                 150                 155                 160

Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
                165                 170                 175

Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
            180                 185                 190

Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
        195                 200                 205

Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
    210                 215                 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
225                 230                 235                 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
                245                 250                 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
            260                 265                 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
        275                 280                 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
    290                 295                 300

Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
305                 310                 315                 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
                325                 330                 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
            340                 345                 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
        355                 360                 365
```

```
Leu Met Thr Val Ala Leu Leu Leu Glu Arg Val Pro Ala Met Ser
    370                 375                 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
385                 390                 395                 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
                405                 410                 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
            420                 425                 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
        435                 440                 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
    450                 455                 460

Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465                 470                 475                 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu
                485                 490                 495

Val Lys Pro Ser Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
            500                 505                 510
```

<210> 6
<211> 512
<212> PRT
<213> HA tagged GLUT4 TAIL mutant

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MPSGFQQIGSEDGEPPQQRVTGTLVLAVFSAVLGSLQFGYNIGVINAPQKVIEQSYNETWLGRQGPEIDYPYDVPDYAE
GPSSIPPGTLTTLWALSVAIFSVGGMISSFLIGIISQWLGRKRAMLVNNVLAVLGGSLMGLANAAASYEMLILGRFLIG
AYSGLTSGLVPMYVGEIAPTHLRGALGTLNQLAIVIGILIAQVLGLESLLGTASLWPLLLGLTVLPALLQLVLLPFCPE
SPRYLYIIQNLEGPARKSLKRLTGWADVSGVLAELKDEKRKLERERPLSLLQLLGSRTHRQPLIIAVVLQLSQQLSGIN
AVFYYSTSIFETAGVGQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLAGMCGCAILMTVALLLLERVPAMSYV
SIVAIFGFVAFFEIGPGPIPWFIVAELFSQGPRPAAMAVAGFSNWTSNFIIGMGFQYVAEAMGPYVFLLFAVLLLGFFI
FTFLRVPETRGRTFDQISAAFHRTPSLLEQEVKPSSIEPAKETTTNV
```

<seq007;prt/1;GLUT4 L489,490A mutant

<400> 6

```
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45
```

```
Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
65                  70                  75                  80

Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
                85                  90                  95

Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
            100                 105                 110

Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
        115                 120                 125

Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
    130                 135                 140

Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
145                 150                 155                 160

Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
                165                 170                 175

Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
            180                 185                 190

Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
        195                 200                 205

Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
    210                 215                 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
225                 230                 235                 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
                245                 250                 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
            260                 265                 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
        275                 280                 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
    290                 295                 300
```

```
Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
305                 310                 315                 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
                325                 330                 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
            340                 345                 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
        355                 360                 365

Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser
    370                 375                 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
385                 390                 395                 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
                405                 410                 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
            420                 425                 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
        435                 440                 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
    450                 455                 460

Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465                 470                 475                 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Ala Ala Glu Gln Glu
                485                 490                 495

Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
            500                 505                 510
```

<210> 7
<211> 521
<212> PRT
<213> HA tagged GLUT4 L489,490A mutant

<400> 7

```
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15
```

```
Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
65                  70                  75                  80

Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
                85                  90                  95

Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
            100                 105                 110

Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
        115                 120                 125

Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
    130                 135                 140

Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
145                 150                 155                 160

Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
                165                 170                 175

Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
            180                 185                 190

Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
        195                 200                 205

Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
    210                 215                 220

Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
225                 230                 235                 240

Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
                245                 250                 255

Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu
```

260 265 270

Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu
275 280 285

Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val
290 295 300

Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
305 310 315 320

Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr
325 330 335

Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser
340 345 350

Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly
355 360 365

Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu
370 375 380

Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile
385 390 395 400

Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
405 410 415

Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
420 425 430

Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
435 440 445

Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
450 455 460

Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
465 470 475 480

Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
485 490 495

Arg Thr Pro Ser Ala Ala Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
500 505 510

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Glu Tyr Leu Gly Pro Asp Glu Asn Asp
        515                 520


<210>  8
<211>  512
<212>  PRT
<213>  GLUT4 F5A mutant

<400>  8

Met Pro Ser Gly Ala Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15


Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30


Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45


Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60


Gly Pro Glu Ile Asp Glu Gly Pro Ser Ser Ile Pro Pro Gly Thr Leu
65                  70                  75                  80


Thr Thr Leu Trp Ala Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met
                85                  90                  95


Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys
            100                 105                 110


Arg Ala Met Leu Val Asn Asn Val Leu Ala Val Leu Gly Gly Ser Leu
        115                 120                 125


Met Gly Leu Ala Asn Ala Ala Ala Ser Tyr Glu Met Leu Ile Leu Gly
    130                 135                 140


Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro
145                 150                 155                 160


Met Tyr Val Gly Glu Ile Ala Pro Thr His Leu Arg Gly Ala Leu Gly
                165                 170                 175


Thr Leu Asn Gln Leu Ala Ile Val Ile Gly Ile Leu Ile Ala Gln Val
            180                 185                 190


Leu Gly Leu Glu Ser Leu Leu Gly Thr Ala Ser Leu Trp Pro Leu Leu
        195                 200                 205
```

```
Leu Gly Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Val Leu Leu Pro
    210                 215                 220

Phe Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Gln Asn Leu Glu
225                 230                 235                 240

Gly Pro Ala Arg Lys Ser Leu Lys Arg Leu Thr Gly Trp Ala Asp Val
                245                 250                 255

Ser Gly Val Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg
            260                 265                 270

Glu Arg Pro Leu Ser Leu Leu Gln Leu Leu Gly Ser Arg Thr His Arg
        275                 280                 285

Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser
    290                 295                 300

Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Thr Ala
305                 310                 315                 320

Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn
                325                 330                 335

Thr Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg
            340                 345                 350

Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly Cys Ala Ile
        355                 360                 365

Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser
    370                 375                 380

Tyr Val Ser Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Ile
385                 390                 395                 400

Gly Pro Gly Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln
                405                 410                 415

Gly Pro Arg Pro Ala Ala Met Ala Val Ala Gly Phe Ser Asn Trp Thr
            420                 425                 430

Ser Asn Phe Ile Ile Gly Met Gly Phe Gln Tyr Val Ala Glu Ala Met
        435                 440                 445

Gly Pro Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe
    450                 455                 460
```

```
Ile Phe Thr Phe Leu Arg Val Pro Glu Thr Arg Gly Arg Thr Phe Asp
465                 470                 475                 480

Gln Ile Ser Ala Ala Phe His Arg Thr Pro Ser Leu Leu Glu Gln Glu
                485                 490                 495

Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu Gly Pro Asp Glu Asn Asp
            500                 505                 510
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<210> 9
<211> 521
<212> PRT
<213> HA tagged GLUT4 F5A mutant

<400> 9

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Met Pro Ser Gly Ala Gln Gln Ile Gly Ser Glu Asp Gly Glu Pro Pro
1               5                   10                  15

Gln Gln Arg Val Thr Gly Thr Leu Val Leu Ala Val Phe Ser Ala Val
            20                  25                  30

Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro
        35                  40                  45

Gln Lys Val Ile Glu Gln Ser Tyr Asn Glu Thr Trp Leu Gly Arg Gln
    50                  55                  60

Gly Pro Glu Ile Asp Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Glu Gly
65                  70                  75                  80

Pro Ser Ser Ile Pro Pro Gly Thr Leu Thr Thr Leu Trp Ala Leu Ser
                85                  90                  95

Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly
            100                 105                 110

Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg Ala Met Leu Val Asn Asn
        115                 120                 125

Val Leu Ala Val Leu Gly Gly Ser Leu Met Gly Leu Ala Asn Ala Ala
    130                 135                 140

Ala Ser Tyr Glu Met Leu Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr
145                 150                 155                 160

Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala
                165                 170                 175
```

```
Pro Thr His Leu Arg Gly Ala Leu Gly Thr Leu Asn Gln Leu Ala Ile
            180                 185                 190

Val Ile Gly Ile Leu Ile Ala Gln Val Leu Gly Leu Glu Ser Leu Leu
        195                 200                 205

Gly Thr Ala Ser Leu Trp Pro Leu Leu Leu Gly Leu Thr Val Leu Pro
    210                 215                 220

Ala Leu Leu Gln Leu Val Leu Leu Pro Phe Cys Pro Glu Ser Pro Arg
225                 230                 235                 240

Tyr Leu Tyr Ile Ile Gln Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu
                245                 250                 255

Lys Arg Leu Thr Gly Trp Ala Asp Val Ser Gly Val Leu Ala Glu Leu
            260                 265                 270

Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Leu Ser Leu Leu
        275                 280                 285

Gln Leu Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val
    290                 295                 300

Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
305                 310                 315                 320

Tyr Ser Thr Ser Ile Phe Glu Thr Ala Gly Val Gly Gln Pro Ala Tyr
                325                 330                 335

Ala Thr Ile Gly Ala Gly Val Val Asn Thr Val Phe Thr Leu Val Ser
            340                 345                 350

Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly
        355                 360                 365

Leu Ala Gly Met Cys Gly Cys Ala Ile Leu Met Thr Val Ala Leu Leu
    370                 375                 380

Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile
385                 390                 395                 400

Phe Gly Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
                405                 410                 415

Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
            420                 425                 430
```

```
Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Ile Gly Met
        435                 440                 445

Gly Phe Gln Tyr Val Ala Glu Ala Met Gly Pro Tyr Val Phe Leu Leu
    450                 455                 460

Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Arg Val
465                 470                 475                 480

Pro Glu Thr Arg Gly Arg Thr Phe Asp Gln Ile Ser Ala Ala Phe His
                485                 490                 495

Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu
            500                 505                 510

Glu Tyr Leu Gly Pro Asp Glu Asn Asp
        515                 520
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<210> 10
<211> 2856
<212> DNA
<213> GLUT1

<220>
<221> CDS
<222> (180)..(1658)
<223>

<400> 10

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tagtcgcggg tccccgagtg agcacgccag ggagcaggag accaaacgac gggggtcgga     60

gtcagagtcg cagtgggagt ccccggaccg gagcacgagc ctgagcggga gagcgccgct    120

cgcacgcccg tcgccacccg cgtacccggc gcagccagag ccaccagcgc agcgctgcc     179

atg gag ccc agc agc aag aag ctg acg ggt cgc ctc atg ctg gct gtg      227
Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1               5                   10                  15

gga gga gca gtg ctt ggc tcc ctg cag ttt ggc tac aac act gga gtc      275
Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
            20                  25                  30

atc aat gcc ccc cag aag gtg atc gag gag ttc tac aac cag aca tgg      323
Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
        35                  40                  45

gtc cac cgc tat ggg gag agc atc ctg ccc acc acg ctc acc acg ctc      371
Val His Arg Tyr Gly Glu Ser Ile Leu Pro Thr Thr Leu Thr Thr Leu
    50                  55                  60

tgg tcc ctc tca gtg gcc atc ttt tct gtt ggg ggc atg att ggc tcc      419
Trp Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser
65                  70                  75                  80
```

```
ttc tct gtg ggc ctt ttc gtt aac cgc ttt ggc cgg cgg aat tca atg      467
Phe Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met
                85                  90                  95

ctg atg atg aac ctg ctg gcc ttc gtg tcc gcc gtg ctc atg ggc ttc      515
Leu Met Met Asn Leu Leu Ala Phe Val Ser Ala Val Leu Met Gly Phe
            100                 105                 110

tcg aaa ctg ggc aag tcc ttt gag atg ctg atc ctg ggc cgc ttc atc      563
Ser Lys Leu Gly Lys Ser Phe Glu Met Leu Ile Leu Gly Arg Phe Ile
        115                 120                 125

atc ggt gtg tac tgc ggc ctg acc aca ggc ttc gtg ccc atg tat gtg      611
Ile Gly Val Tyr Cys Gly Leu Thr Thr Gly Phe Val Pro Met Tyr Val
    130                 135                 140

ggt gaa gtg tca ccc aca gcc ttt cgt ggg gcc ctg ggc acc ctg cac      659
Gly Glu Val Ser Pro Thr Ala Phe Arg Gly Ala Leu Gly Thr Leu His
145                 150                 155                 160

cag ctg ggc atc gtc gtc ggc atc ctc atc gcc cag gtg ttc ggc ctg      707
Gln Leu Gly Ile Val Val Gly Ile Leu Ile Ala Gln Val Phe Gly Leu
                165                 170                 175

gac tcc atc atg ggc aac aag gac ctg tgg ccc ctg ctg ctg agc atc      755
Asp Ser Ile Met Gly Asn Lys Asp Leu Trp Pro Leu Leu Leu Ser Ile
            180                 185                 190

atc ttc atc ccg gcc ctg ctg cag tgc atc gtg ctg ccc ttc tgc ccc      803
Ile Phe Ile Pro Ala Leu Leu Gln Cys Ile Val Leu Pro Phe Cys Pro
        195                 200                 205

gag agt ccc cgc ttc ctg ctc atc aac cgc aac gag gag aac cgg gcc      851
Glu Ser Pro Arg Phe Leu Leu Ile Asn Arg Asn Glu Glu Asn Arg Ala
    210                 215                 220

aag agt gtg cta aag aag ctg cgc ggg aca gct gac gtg acc cat gac      899
Lys Ser Val Leu Lys Lys Leu Arg Gly Thr Ala Asp Val Thr His Asp
225                 230                 235                 240

ctg cag gag atg aag gaa gag agt cgg cag atg atg cgg gag aag aag      947
Leu Gln Glu Met Lys Glu Glu Ser Arg Gln Met Met Arg Glu Lys Lys
                245                 250                 255

gtc acc atc ctg gag ctg ttc cgc tcc ccc gcc tac cgc cag ccc atc      995
Val Thr Ile Leu Glu Leu Phe Arg Ser Pro Ala Tyr Arg Gln Pro Ile
            260                 265                 270

ctc atc gct gtg gtg ctg cag ctg tcc cag cag ctg tct ggc atc aac     1043
Leu Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn
        275                 280                 285

gct gtc ttc tat tac tcc acg agc atc ttc gag aag gcg ggg gtg cag     1091
Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Lys Ala Gly Val Gln
    290                 295                 300

cag cct gtg tat gcc acc att ggc tcc ggt atc gtc aac acg gcc ttc     1139
Gln Pro Val Tyr Ala Thr Ile Gly Ser Gly Ile Val Asn Thr Ala Phe
305                 310                 315                 320

act gtc gtg tcg ctg ttt gtg gtg gag cga gca ggc cgg cgg acc ctg     1187
Thr Val Val Ser Leu Phe Val Val Glu Arg Ala Gly Arg Arg Thr Leu
                325                 330                 335
```

```
cac ctc ata ggc ctc gct ggc atg gcg ggt tgt gcc ata ctc atg acc     1235
His Leu Ile Gly Leu Ala Gly Met Ala Gly Cys Ala Ile Leu Met Thr
            340                 345                 350

atc gcg cta gca ctg ctg gag cag cta ccc tgg atg tcc tat ctg agc     1283
Ile Ala Leu Ala Leu Leu Glu Gln Leu Pro Trp Met Ser Tyr Leu Ser
        355                 360                 365

atc gtg gcc atc ttt ggc ttt gtg gcc ttc ttt gaa gtg ggt cct ggc     1331
Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Val Gly Pro Gly
    370                 375                 380

ccc atc cca tgg ttc atc gtg gct gaa ctc ttc agc cag ggt cca cgt     1379
Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg
385                 390                 395                 400

cca gct gcc att gcc gtt gca ggc ttc tcc aac tgg acc tca aat ttc     1427
Pro Ala Ala Ile Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe
                405                 410                 415

att gtg ggc atg tgc ttc cag tat gtg gag caa ctg tgt ggt ccc tac     1475
Ile Val Gly Met Cys Phe Gln Tyr Val Glu Gln Leu Cys Gly Pro Tyr
            420                 425                 430

gtc ttc atc atc ttc act gtg ctc ctg gtt ctg ttc ttc atc ttc acc     1523
Val Phe Ile Ile Phe Thr Val Leu Leu Val Leu Phe Phe Ile Phe Thr
        435                 440                 445

tac ttc aaa gtt cct gag act aaa ggc cgg acc ttc gat gag atc gct     1571
Tyr Phe Lys Val Pro Glu Thr Lys Gly Arg Thr Phe Asp Glu Ile Ala
    450                 455                 460

tcc ggc ttc cgg cag ggg gga gcc agc caa agt gat aag aca ccc gag     1619
Ser Gly Phe Arg Gln Gly Gly Ala Ser Gln Ser Asp Lys Thr Pro Glu
465                 470                 475                 480

gag ctg ttc cat ccc ctg ggg gct gat tcc caa gtg tga gtcgccccag      1668
Glu Leu Phe His Pro Leu Gly Ala Asp Ser Gln Val
                485                 490

atcaccagcc cggcctgctc ccagcagccc taaggatctc tcaggagcac aggcagctgg   1728

atgagacttc caaacctgac agatgtcagc cgagccgggc ctggggctcc tttctccagc   1788

cagcaatgat gtccagaaga atattcagga cttaacggct ccaggatttt aacaaaagca   1848

agactgttgc tcaaatctat tcagacaagc aacaggtttt ataatttttt tattactgat   1908

tttgttattt ttatatcagc ctgagtctcc tgtgcccaca tcccaggctt caccctgaat   1968

ggttccatgc ctgagggtgg agactaagcc ctgtcgagac acttgccttc ttcacccagc   2028

taatctgtag ggctggacct atgtcctaag gacacactaa tcgaactatg aactacaaag   2088

cttctatccc aggaggtggc tatggccacc cgttctgctg gcctggatct ccccactcta   2148

ggggtcaggc tccattagga tttgcccctt cccatctctt cctacccaac cactcaaatt   2208

aatctttctt tacctgagac cagttgggag cactggagtg cagggaggag aggggaaggg   2268.

ccagtctggg ctgccgggtt ctagtctcct ttgcactgag ggccacacta ttaccatgag   2328
```

aagagggcct gtgggagcct gcaaactcac tgctcaagaa gacatggaga ctcctgccct 2388

gttgtgtata gatgcaagat atttatatat atttttggtt gtcaatatta aatacagaca 2448

ctaagttata gtatatctgg acaagccaac ttgtaaatac accacctcac tcctgttact 2508

tacctaaaca gatataaatg gctggttttt agaaacatgg ttttgaaatg cttgtggatt 2568

gagggtagga ggtttggatg ggagtgagac agaagtaagt ggggttgcaa ccactgcaac 2628

ggcttagact tcgactcagg atccagtccc ttacacgtac ctctcatcag tgtcctcttg 2688

ctcaaaaatc tgtttgatcc ctgttaccca gagaatatat acattcttta tcttgacatt 2748

caaggcattt ctatcacata tttgatagtt ggtgttcaaa aaaacactag ttttgtgcca 2808

gccgtgatgc tcaggcttga aatcgcatta ttttgaatgt gaagggaa 2856

<210> 11
<211> 492
<212> PRT
<213> GLUT1

<400> 11

Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1 5 10 15

Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
20 25 30

Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
35 40 45

Val His Arg Tyr Gly Glu Ser Ile Leu Pro Thr Thr Leu Thr Thr Leu
50 55 60

Trp Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser
65 70 75 80

Phe Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met
85 90 95

Leu Met Met Asn Leu Leu Ala Phe Val Ser Ala Val Leu Met Gly Phe
100 105 110

Ser Lys Leu Gly Lys Ser Phe Glu Met Leu Ile Leu Gly Arg Phe Ile
115 120 125

Ile Gly Val Tyr Cys Gly Leu Thr Thr Gly Phe Val Pro Met Tyr Val
130 135 140

Gly Glu Val Ser Pro Thr Ala Phe Arg Gly Ala Leu Gly Thr Leu His

```
145                 150                 155                 160

Gln Leu Gly Ile Val Val Gly Ile Leu Ile Ala Gln Val Phe Gly Leu
                165                 170                 175

Asp Ser Ile Met Gly Asn Lys Asp Leu Trp Pro Leu Leu Leu Ser Ile
            180                 185                 190

Ile Phe Ile Pro Ala Leu Leu Gln Cys Ile Val Leu Pro Phe Cys Pro
        195                 200                 205

Glu Ser Pro Arg Phe Leu Leu Ile Asn Arg Asn Glu Glu Asn Arg Ala
    210                 215                 220

Lys Ser Val Leu Lys Lys Leu Arg Gly Thr Ala Asp Val Thr His Asp
225                 230                 235                 240

Leu Gln Glu Met Lys Glu Glu Ser Arg Gln Met Met Arg Glu Lys Lys
                245                 250                 255

Val Thr Ile Leu Glu Leu Phe Arg Ser Pro Ala Tyr Arg Gln Pro Ile
            260                 265                 270

Leu Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn
        275                 280                 285

Ala Val Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Lys Ala Gly Val Gln
    290                 295                 300

Gln Pro Val Tyr Ala Thr Ile Gly Ser Gly Ile Val Asn Thr Ala Phe
305                 310                 315                 320

Thr Val Val Ser Leu Phe Val Val Glu Arg Ala Gly Arg Arg Thr Leu
                325                 330                 335

His Leu Ile Gly Leu Ala Gly Met Ala Gly Cys Ala Ile Leu Met Thr
            340                 345                 350

Ile Ala Leu Ala Leu Leu Glu Gln Leu Pro Trp Met Ser Tyr Leu Ser
        355                 360                 365

Ile Val Ala Ile Phe Gly Phe Val Ala Phe Phe Glu Val Gly Pro Gly
    370                 375                 380

Pro Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg
385                 390                 395                 400
```

```
Pro Ala Ala Ile Ala Val Ala Gly Phe Ser Asn Trp Thr Ser Asn Phe
                405                 410                 415

Ile Val Gly Met Cys Phe Gln Tyr Val Glu Gln Leu Cys Gly Pro Tyr
            420                 425                 430

Val Phe Ile Ile Phe Thr Val Leu Leu Val Leu Phe Phe Ile Phe Thr
        435                 440                 445

Tyr Phe Lys Val Pro Glu Thr Lys Gly Arg Thr Phe Asp Glu Ile Ala
    450                 455                 460

Ser Gly Phe Arg Gln Gly Gly Ala Ser Gln Ser Asp Lys Thr Pro Glu
465                 470                 475                 480

Glu Leu Phe His Pro Leu Gly Ala Asp Ser Gln Val
                485                 490
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<210> 12
<211> 1506
<212> DNA
<213> HA tagged GLUT1

<220>
<221> CDS
<222> (1)..(1506)
<223>

<400> 12

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atg gag ccc agc agc aag aag ctg acg ggt cgc ctc atg ctg gct gtg       48
Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1               5                   10                  15

gga gga gca gtg ctt ggc tcc ctg cag ttt ggc tac aac act gga gtc       96
Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
            20                  25                  30

atc aat gcc ccc cag aag gtg atc gag gag ttc tac aac cag aca tgg      144
Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
        35                  40                  45

gtc cac cgc tat ggg gag agc atc tac cca tac gac gtc cca gac tac      192
Val His Arg Tyr Gly Glu Ser Ile Tyr Pro Tyr Asp Val Pro Asp Tyr
    50                  55                  60

gct ctg ccc acc acg ctc acc acg ctc tgg tcc ctc tca gtg gcc atc      240
Ala Leu Pro Thr Thr Leu Thr Thr Leu Trp Ser Leu Ser Val Ala Ile
65                  70                  75                  80

ttt tct gtt ggg ggc atg att ggc tcc ttc tct gtg ggc ctt ttc gtt      288
Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly Leu Phe Val
                85                  90                  95

aac cgc ttt ggc cgg cgg aat tca atg ctg atg atg aac ctg ctg gcc      336
Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Met Met Asn Leu Leu Ala
            100                 105                 110
```

```
ttc gtg tcc gcc gtg ctc atg ggc ttc tcg aaa ctg ggc aag tcc ttt      384
Phe Val Ser Ala Val Leu Met Gly Phe Ser Lys Leu Gly Lys Ser Phe
        115                 120                 125

gag atg ctg atc ctg ggc cgc ttc atc atc ggt gtg tac tgc ggc ctg      432
Glu Met Leu Ile Leu Gly Arg Phe Ile Ile Gly Val Tyr Cys Gly Leu
    130                 135                 140

acc aca ggc ttc gtg ccc atg tat gtg ggt gaa gtg tca ccc aca gcc      480
Thr Thr Gly Phe Val Pro Met Tyr Val Gly Glu Val Ser Pro Thr Ala
145                 150                 155                 160

ttt cgt ggg gcc ctg ggc acc ctg cac cag ctg ggc atc gtc gtc ggc      528
Phe Arg Gly Ala Leu Gly Thr Leu His Gln Leu Gly Ile Val Val Gly
                165                 170                 175

atc ctc atc gcc cag gtg ttc ggc ctg gac tcc atc atg ggc aac aag      576
Ile Leu Ile Ala Gln Val Phe Gly Leu Asp Ser Ile Met Gly Asn Lys
            180                 185                 190

gac ctg tgg ccc ctg ctg ctg agc atc atc ttc atc ccg gcc ctg ctg      624
Asp Leu Trp Pro Leu Leu Leu Ser Ile Ile Phe Ile Pro Ala Leu Leu
        195                 200                 205

cag tgc atc gtg ctg ccc ttc tgc ccc gag agt ccc cgc ttc ctg ctc      672
Gln Cys Ile Val Leu Pro Phe Cys Pro Glu Ser Pro Arg Phe Leu Leu
    210                 215                 220

atc aac cgc aac gag gag aac cgg gcc aag agt gtg cta aag aag ctg      720
Ile Asn Arg Asn Glu Glu Asn Arg Ala Lys Ser Val Leu Lys Lys Leu
225                 230                 235                 240

cgc ggg aca gct gac gtg acc cat gac ctg cag gag atg aag gaa gag      768
Arg Gly Thr Ala Asp Val Thr His Asp Leu Gln Glu Met Lys Glu Glu
                245                 250                 255

agt cgg cag atg atg cgg gag aag aag gtc acc atc ctg gag ctg ttc      816
Ser Arg Gln Met Met Arg Glu Lys Lys Val Thr Ile Leu Glu Leu Phe
            260                 265                 270

cgc tcc ccc gcc tac cgc cag ccc atc ctc atc gct gtg gtg ctg cag      864
Arg Ser Pro Ala Tyr Arg Gln Pro Ile Leu Ile Ala Val Val Leu Gln
        275                 280                 285

ctg tcc cag cag ctg tct ggc atc aac gct gtc ttc tat tac tcc acg      912
Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr
    290                 295                 300

agc atc ttc gag aag gcg ggg gtg cag cag cct gtg tat gcc acc att      960
Ser Ile Phe Glu Lys Ala Gly Val Gln Gln Pro Val Tyr Ala Thr Ile
305                 310                 315                 320

ggc tcc ggt atc gtc aac acg gcc ttc act gtc gtg tcg ctg ttt gtg     1008
Gly Ser Gly Ile Val Asn Thr Ala Phe Thr Val Val Ser Leu Phe Val
                325                 330                 335

gtg gag cga gca ggc cgg cgg acc ctg cac ctc ata ggc ctc gct ggc     1056
Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Ile Gly Leu Ala Gly
            340                 345                 350

atg gcg ggt tgt gcc ata ctc atg acc atc gcg cta gca ctg ctg gag     1104
Met Ala Gly Cys Ala Ile Leu Met Thr Ile Ala Leu Ala Leu Leu Glu
        355                 360                 365
```

```
cag cta ccc tgg atg tcc tat ctg agc atc gtg gcc atc ttt ggc ttt     1152
Gln Leu Pro Trp Met Ser Tyr Leu Ser Ile Val Ala Ile Phe Gly Phe
    370                 375                 380

gtg gcc ttc ttt gaa gtg ggt cct ggc ccc atc cca tgg ttc atc gtg     1200
Val Ala Phe Phe Glu Val Gly Pro Gly Pro Ile Pro Trp Phe Ile Val
385                 390                 395                 400

gct gaa ctc ttc agc cag ggt cca cgt cca gct gcc att gcc gtt gca     1248
Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Ile Ala Val Ala
                405                 410                 415

ggc ttc tcc aac tgg acc tca aat ttc att gtg ggc atg tgc ttc cag     1296
Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Val Gly Met Cys Phe Gln
            420                 425                 430

tat gtg gag caa ctg tgt ggt ccc tac gtc ttc atc atc ttc act gtg     1344
Tyr Val Glu Gln Leu Cys Gly Pro Tyr Val Phe Ile Ile Phe Thr Val
        435                 440                 445

ctc ctg gtt ctg ttc ttc atc ttc acc tac ttc aaa gtt cct gag act     1392
Leu Leu Val Leu Phe Phe Ile Phe Thr Tyr Phe Lys Val Pro Glu Thr
    450                 455                 460

aaa ggc cgg acc ttc gat gag atc gct tcc ggc ttc cgg cag ggg gga     1440
Lys Gly Arg Thr Phe Asp Glu Ile Ala Ser Gly Phe Arg Gln Gly Gly
465                 470                 475                 480

gcc agc caa agt gat aag aca ccc gag gag ctg ttc cat ccc ctg ggg     1488
Ala Ser Gln Ser Asp Lys Thr Pro Glu Glu Leu Phe His Pro Leu Gly
                485                 490                 495

gct gat tcc caa gtg tga                                             1506
Ala Asp Ser Gln Val
            500
```

<210> 13
<211> 501
<212> PRT
<213> HA tagged GLUT1

<400> 13

```
Met Glu Pro Ser Ser Lys Lys Leu Thr Gly Arg Leu Met Leu Ala Val
1               5                   10                  15

Gly Gly Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Thr Gly Val
            20                  25                  30

Ile Asn Ala Pro Gln Lys Val Ile Glu Glu Phe Tyr Asn Gln Thr Trp
        35                  40                  45

Val His Arg Tyr Gly Glu Ser Ile Tyr Pro Tyr Asp Val Pro Asp Tyr
    50                  55                  60

Ala Leu Pro Thr Thr Leu Thr Thr Leu Trp Ser Leu Ser Val Ala Ile
65                  70                  75                  80
```

```
Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly Leu Phe Val
                85                  90                  95

Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Met Met Asn Leu Leu Ala
            100                 105                 110

Phe Val Ser Ala Val Leu Met Gly Phe Ser Lys Leu Gly Lys Ser Phe
        115                 120                 125

Glu Met Leu Ile Leu Gly Arg Phe Ile Ile Gly Val Tyr Cys Gly Leu
    130                 135                 140

Thr Thr Gly Phe Val Pro Met Tyr Val Gly Glu Val Ser Pro Thr Ala
145                 150                 155                 160

Phe Arg Gly Ala Leu Gly Thr Leu His Gln Leu Gly Ile Val Val Gly
                165                 170                 175

Ile Leu Ile Ala Gln Val Phe Gly Leu Asp Ser Ile Met Gly Asn Lys
            180                 185                 190

Asp Leu Trp Pro Leu Leu Leu Ser Ile Ile Phe Ile Pro Ala Leu Leu
        195                 200                 205

Gln Cys Ile Val Leu Pro Phe Cys Pro Glu Ser Pro Arg Phe Leu Leu
    210                 215                 220

Ile Asn Arg Asn Glu Glu Asn Arg Ala Lys Ser Val Leu Lys Lys Leu
225                 230                 235                 240

Arg Gly Thr Ala Asp Val Thr His Asp Leu Gln Glu Met Lys Glu Glu
                245                 250                 255

Ser Arg Gln Met Met Arg Glu Lys Lys Val Thr Ile Leu Glu Leu Phe
            260                 265                 270

Arg Ser Pro Ala Tyr Arg Gln Pro Ile Leu Ile Ala Val Val Leu Gln
        275                 280                 285

Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr Tyr Ser Thr
    290                 295                 300

Ser Ile Phe Glu Lys Ala Gly Val Gln Gln Pro Val Tyr Ala Thr Ile
305                 310                 315                 320

Gly Ser Gly Ile Val Asn Thr Ala Phe Thr Val Val Ser Leu Phe Val
```

325 330 335

Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Ile Gly Leu Ala Gly
340 345 350

Met Ala Gly Cys Ala Ile Leu Met Thr Ile Ala Leu Ala Leu Leu Glu
355 360 365

Gln Leu Pro Trp Met Ser Tyr Leu Ser Ile Val Ala Ile Phe Gly Phe
370 375 380

Val Ala Phe Phe Glu Val Gly Pro Gly Pro Ile Pro Trp Phe Ile Val
385 390 395 400

Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Ile Ala Val Ala
405 410 415

Gly Phe Ser Asn Trp Thr Ser Asn Phe Ile Val Gly Met Cys Phe Gln
420 425 430

Tyr Val Glu Gln Leu Cys Gly Pro Tyr Val Phe Ile Ile Phe Thr Val
435 440 445

Leu Leu Val Leu Phe Phe Ile Phe Thr Tyr Phe Lys Val Pro Glu Thr
450 455 460

Lys Gly Arg Thr Phe Asp Glu Ile Ala Ser Gly Phe Arg Gln Gly Gly
465 470 475 480

Ala Ser Gln Ser Asp Lys Thr Pro Glu Glu Leu Phe His Pro Leu Gly
485 490 495

Ala Asp Ser Gln Val
500

<210> 14
<211> 9
<212> PRT
<213> HA epitope

<400> 14

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5

<210> 15
<211> 14
<212> PRT
<213> Simian Virus 5 epitope (SV5)

<400> 15

Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr
1 5 10

<210> 16
<211> 6
<212> PRT
<213> hexa-his

<400> 16

His His His His His His
1 5

<210> 17
<211> 10
<212> PRT
<213> c-myc epitope

<400> 17

Phe Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

<210> 18
<211> 9
<212> PRT
<213> FLAG epitope

<400> 18

Asp Tyr Lys Asp Asp Asp Asp Lys Cys
1 5

<210> 19
<211> 9
<212> PRT
<213> Alternative FLAG epitope

<400> 19

Met Asp Phe Lys Asp Asp Asp Asp Lys
1 5

<210> 20
<211> 9
<212> PRT
<213> Alternative FLAG epitope

<400> 20

Met Asp Tyr Lys Ala Phe Asp Asn Leu
1 5

<210> 21
<211> 223

<212> PRT
<213> glutathione-S-transferase

<400> 21

Met Ala Lys Leu Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val
1 5 10 15

Gln Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu
20 25 30

His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe
35 40 45

Glu Leu Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp
50 55 60

Val Lys Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys
65 70 75 80

His Asn Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met
85 90 95

Leu Glu Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala
100 105 110

Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu
115 120 125

Pro Glu Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr
130 135 140

Leu Asn Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala
145 150 155 160

Leu Asp Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro
165 170 175

Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp
180 185 190

Lys Tyr Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp
195 200 205

Gln Ala Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu
210 215 220

<210> 22
<211> 488

<212> PRT
<213> maltose binding protein

<400> 22

Met Lys Ile Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240

```
Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
                245                 250                 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
            260                 265                 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
        275                 280                 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
    290                 295                 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305                 310                 315                 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
                325                 330                 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
            340                 345                 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
        355                 360                 365

Ser Ser Ser Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Leu Gly Ile
    370                 375                 380

Asp Thr Thr Glu Asn Leu Tyr Phe Gln Gly Ala Met Asp Pro Glu Phe
385                 390                 395                 400

Lys Gly Leu Arg Arg Arg Ala Gln Leu Val Arg Pro Leu Ser Asn Leu
                405                 410                 415

Glu Pro Ala Val Ser Arg His Ala Val Pro Ser Leu Ala Leu Ala Val
            420                 425                 430

Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu Asn
        435                 440                 445

Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu Glu
    450                 455                 460

Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu
465                 470                 475                 480

Trp Gln Leu Gly Cys Phe Gly Gly
```

485

<210> 23
<211> 168
<212> PRT
<213> GAL4

<400> 23

Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
130 135 140

Thr Val Ser Pro Glu Phe Pro Gly Ile Arg Arg Leu Asp Ala Leu Ile
145 150 155 160

Ser Ser Arg Ala Ala Ala Gly Thr
165

<210> 24
<211> 1045
<212> PRT
<213> beta-galactosidase

<400> 24

Met Ser Phe Thr Leu Thr Asn Lys Asn Val Ile Phe Val Ala Gly Leu
1 5 10 15

```
Gly Gly Ile Gly Leu Asp Thr Ser Lys Glu Leu Leu Lys Arg Asp Pro
            20                  25                  30

Val Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu
        35                  40                  45

Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu
    50                  55                  60

Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly
65                  70                  75                  80

Glu Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Glu Ser
                85                  90                  95

Trp Leu Glu Cys Asp Leu Pro Glu Ala Asp Thr Val Val Val Pro Ser
            100                 105                 110

Asn Trp Gln Met His Gly Tyr Asp Ala Pro Ile Tyr Thr Asn Val Thr
        115                 120                 125

Tyr Pro Ile Thr Val Asn Pro Pro Phe Val Pro Thr Glu Asn Pro Thr
    130                 135                 140

Gly Cys Tyr Ser Leu Thr Phe Asn Val Asp Glu Ser Trp Leu Gln Glu
145                 150                 155                 160

Gly Gln Thr Arg Ile Ile Phe Asp Gly Val Asn Ser Ala Phe His Leu
                165                 170                 175

Trp Cys Asn Gly Arg Trp Val Gly Tyr Gly Gln Asp Ser Arg Leu Pro
            180                 185                 190

Ser Glu Phe Asp Leu Ser Ala Phe Leu Arg Ala Gly Glu Asn Arg Leu
        195                 200                 205

Ala Val Met Val Leu Arg Trp Ser Asp Gly Ser Tyr Leu Glu Asp Gln
    210                 215                 220

Asp Met Trp Arg Met Ser Gly Ile Phe Arg Asp Val Ser Leu Leu His
225                 230                 235                 240

Lys Pro Thr Thr Gln Ile Ser Asp Phe His Val Ala Thr Arg Phe Asn
                245                 250                 255

Asp Asp Phe Ser Arg Ala Val Leu Glu Ala Glu Val Gln Met Cys Gly
```

260 265 270

Glu Leu Arg Asp Tyr Leu Arg Val Thr Val Ser Leu Trp Gln Gly Glu
275 280 285

Thr Gln Val Ala Ser Gly Thr Ala Pro Phe Gly Gly Glu Ile Ile Asp
290 295 300

Glu Arg Gly Gly Tyr Ala Asp Arg Val Thr Leu Arg Leu Asn Val Glu
305 310 315 320

Asn Pro Lys Leu Trp Ser Ala Glu Ile Pro Asn Leu Tyr Arg Ala Val
325 330 335

Val Glu Leu His Thr Ala Asp Gly Thr Leu Ile Glu Ala Glu Ala Cys
340 345 350

Asp Val Gly Phe Arg Glu Val Arg Ile Glu Asn Gly Leu Leu Leu Leu
355 360 365

Asn Gly Lys Pro Leu Leu Ile Arg Gly Val Asn Arg His Glu His His
370 375 380

Pro Leu His Gly Gln Val Met Asp Glu Gln Thr Met Val Gln Asp Ile
385 390 395 400

Leu Leu Met Lys Gln Asn Asn Phe Asn Ala Val Arg Cys Ser His Tyr
405 410 415

Pro Asn His Pro Leu Trp Tyr Thr Leu Cys Asp Arg Tyr Gly Leu Tyr
420 425 430

Val Val Asp Glu Ala Asn Ile Glu Thr His Gly Met Val Pro Met Asn
435 440 445

Arg Leu Thr Asp Asp Pro Arg Trp Leu Pro Ala Met Ser Glu Arg Val
450 455 460

Thr Arg Met Val Gln Arg Asp Arg Asn His Pro Ser Val Ile Ile Trp
465 470 475 480

Ser Leu Gly Asn Glu Ser Gly His Gly Ala Asn His Asp Ala Leu Tyr
485 490 495

Arg Trp Ile Lys Ser Val Asp Pro Ser Arg Pro Val Gln Tyr Glu Gly
500 505 510

Gly Gly Ala Asp Thr Thr Ala Thr Asp Ile Ile Cys Pro Met Tyr Ala
515 520 525

Arg Val Asp Glu Asp Gln Pro Phe Pro Ala Val Pro Lys Trp Ser Ile
530 535 540

Lys Lys Trp Leu Ser Leu Pro Gly Glu Thr Arg Pro Leu Ile Leu Cys
545 550 555 560

Glu Tyr Ala His Ala Met Gly Asn Ser Leu Gly Gly Phe Ala Lys Tyr
565 570 575

Trp Gln Ala Phe Arg Gln Tyr Pro Arg Leu Gln Gly Gly Phe Val Trp
580 585 590

Asp Trp Val Asp Gln Ser Leu Ile Lys Tyr Asp Glu Asn Gly Asn Pro
595 600 605

Trp Ser Ala Tyr Gly Gly Asp Phe Gly Asp Thr Pro Asn Asp Arg Gln
610 615 620

Phe Cys Met Asn Gly Leu Val Phe Ala Asp Arg Thr Pro His Pro Ala
625 630 635 640

Leu Thr Glu Ala Lys His Gln Gln Gln Phe Phe Gln Phe Arg Leu Ser
645 650 655

Gly Gln Thr Ile Glu Val Thr Ser Glu Tyr Leu Phe Arg His Ser Asp
660 665 670

Asn Glu Leu Leu His Trp Met Val Ala Leu Asp Gly Lys Pro Leu Ala
675 680 685

Ser Gly Glu Val Pro Leu Asp Val Ala Pro Gln Gly Lys Gln Leu Ile
690 695 700

Glu Leu Pro Glu Leu Pro Gln Pro Glu Ser Ala Gly Gln Leu Trp Leu
705 710 715 720

Thr Val Arg Val Val Gln Pro Asn Ala Thr Ala Trp Ser Glu Ala Gly
725 730 735

His Ile Ser Ala Trp Gln Gln Trp Arg Leu Ala Glu Asn Leu Ser Val
740 745 750

Thr Leu Pro Ala Ala Ser His Ala Ile Pro His Leu Thr Thr Ser Glu
755 760 765

```
Met Asp Phe Cys Ile Glu Leu Gly Asn Lys Arg Trp Gln Phe Asn Arg
    770                 775                 780

Gln Ser Gly Phe Leu Ser Gln Met Trp Ile Gly Asp Lys Lys Gln Leu
785                 790                 795                 800

Leu Thr Pro Leu Arg Asp Gln Phe Thr Arg Ala Pro Leu Asp Asn Asp
                805                 810                 815

Ile Gly Val Ser Glu Ala Thr Arg Ile Asp Pro Asn Ala Trp Val Glu
            820                 825                 830

Arg Trp Lys Ala Ala Gly His Tyr Gln Ala Glu Ala Ala Leu Leu Gln
        835                 840                 845

Cys Thr Ala Asp Thr Leu Ala Asp Ala Val Leu Ile Thr Thr Ala His
    850                 855                 860

Ala Trp Gln His Gln Gly Lys Thr Leu Phe Ile Ser Arg Lys Thr Tyr
865                 870                 875                 880

Arg Ile Asp Gly Ser Gly Gln Met Ala Ile Thr Val Asp Val Glu Val
                885                 890                 895

Ala Ser Asp Thr Pro His Pro Ala Arg Ile Gly Leu Asn Cys Gln Leu
            900                 905                 910

Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu Gly Pro Gln Glu
        915                 920                 925

Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp Arg Trp Asp Leu
    930                 935                 940

Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe Pro Ser Glu Asn Gly
945                 950                 955                 960

Leu Arg Cys Gly Thr Arg Glu Leu Asn Tyr Gly Pro His Gln Trp Arg
                965                 970                 975

Gly Asp Phe Gln Phe Asn Ile Ser Arg Tyr Ser Gln Gln Gln Leu Met
            980                 985                 990

Ser His Arg His Leu Leu His Ala  Glu Glu Gly Thr Trp  Leu Asn Ile
        995                 1000                1005

Asp Gly  Phe His Met Gly Ile  Gly Gly Asp Asp Ser  Trp Ser Pro
    1010                1015                1020
```

Ser Val Ser Ala Glu Leu Gln Leu Ser Ala Gly Arg Tyr His Tyr
1025 1030 1035

Gln Leu Val Trp Cys Gln Lys
1040 1045

<210> 25
<211> 238
<212> PRT
<213> enhanced green fluorescence protein (eGFP)

<400> 25

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Gln
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 26
<211> 264
<212> PRT
<213> yellow fluorescent protein

<400> 26

Met Asp Gly Thr Glu Leu Gly Ser Thr Arg Asp Ser Arg Gly Ser Gly
1 5 10 15

Gly Ser Gly Gly Ser Gly Gly Ser Gly Met Val Ser Lys Gly Glu Glu
20 25 30

Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val
35 40 45

Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr
50 55 60

Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
65 70 75 80

Val Pro Trp Pro Thr Leu Val Thr Thr Phe Gly Tyr Gly Leu Gln Cys
85 90 95

Phe Ala Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser
100 105 110

Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp
115 120 125

Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
130 135 140

Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly
145 150 155 160

Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val
165 170 175

Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys
180 185 190

Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr
195 200 205

Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
210 215 220

His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys
225 230 235 240

Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr
245 250 255

Leu Gly Met Asp Glu Leu Tyr Lys
260

<210> 27
<211> 238
<212> PRT
<213> soluble modified blue fluorescent protein

<400> 27

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

```
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

<210> 28
<211> 238
<212> PRT
<213> soluble-modified red-shifted green fluorescent protein

<400> 28

```
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1               5                   10                  15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95

Thr Ile Ser Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
```

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 29
<211> 262
<212> PRT
<213> cyan fluorescent protein

<400> 29

Met His His His His His His His Asp Gly Thr Met Val Ser Lys Gly
1 5 10 15

Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly
20 25 30

Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp
35 40 45

Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys
50 55 60

Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Trp Gly Val
65 70 75 80

Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe
85 90 95

```
Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe
            100                 105                 110

Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly
        115                 120                 125

Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu
    130                 135                 140

Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Ile Ser His
145                 150                 155                 160

Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn
                165                 170                 175

Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp
            180                 185                 190

His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
        195                 200                 205

Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn
    210                 215                 220

Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
225                 230                 235                 240

Ile Thr Leu Gly Met Asp Glu Leu Tyr Ser Gly Ser Gly Ser Gly Ser
                245                 250                 255

Leu Glu Gly Thr Glu Leu
            260
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<210> 30
<211> 8
<212> PRT
<213> streptavidin binding sequence

<400> 30

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Trp Ser His Pro Gln Phe Glu Lys
1               5
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<210> 31
<211> 574
<212> PRT
<213> strepsolysin-O

<400> 31

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Met Lys Asp Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val
1               5                  10                  15

Ala Gly Leu Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala
            20                  25                  30

Asn Ala Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr
        35                  40                  45

Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys
    50                  55                  60

Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys
65                  70                  75                  80

Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys
                85                  90                  95

Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile
            100                 105                 110

Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala
        115                 120                 125

Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys
    130                 135                 140

Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn
145                 150                 155                 160

Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr
                165                 170                 175

Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys
            180                 185                 190

Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp
        195                 200                 205

Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr
    210                 215                 220

Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His
225                 230                 235                 240

Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr
                245                 250                 255
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Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
            260                 265                 270

Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
        275                 280                 285

Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
    290                 295                 300

Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
305                 310                 315                 320

Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
                325                 330                 335

Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
            340                 345                 350

Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
        355                 360                 365

Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
    370                 375                 380

Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
385                 390                 395                 400

Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
                405                 410                 415

Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
            420                 425                 430

Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
        435                 440                 445

Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
    450                 455                 460

Ser Gly Lys Ile Asn Leu Ser His Arg Gly Ala Tyr Val Ala Gln Tyr
465                 470                 475                 480

Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
                485                 490                 495

Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
```

500 505 510

Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
515 520 525

Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val
530 535 540

Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
545 550 555 560

Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
565 570

<210> 32
<211> 293
<212> PRT
<213> alpha-hemolysin

<400> 32

Ala Asp Ser Asp Ile Asn Ile Lys Thr Gly Thr Thr Asp Ile Gly Ser
1 5 10 15

Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu Asn
20 25 30

Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
35 40 45

Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly Gln
50 55 60

Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp
65 70 75 80

Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val Ala
85 90 95

Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu Tyr
100 105 110

Met Ser Thr Leu Thr Tyr Gly Phe Asn Gly Asn Val Thr Gly Asp Asp
115 120 125

Thr Gly Lys Ile Gly Gly Leu Ile Gly Ala Asn Val Ser Ile Gly His
130 135 140

Thr Leu Lys Tyr Val Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro

145 150 155 160

Thr Asp Lys Lys Val Gly Trp Lys Val Ile Phe Asn Asn Met Val Asn
165 170 175

Gln Asn Trp Gly Pro Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly
180 185 190

Asn Gln Leu Phe Met Lys Thr Arg Asn Gly Ser Met Lys Ala Ala Asp
195 200 205

Asn Phe Leu Asp Pro Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe
210 215 220

Ser Pro Asp Phe Ala Thr Val Ile Thr Met Asp Arg Lys Ala Ser Lys
225 230 235 240

Gln Gln Thr Asn Ile Asp Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr
245 250 255

Gln Leu His Trp Thr Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp
260 265 270

Lys Trp Thr Asp Arg Ser Ser Glu Arg Tyr Lys Ile Asp Trp Glu Lys
275 280 285

Glu Glu Met Thr Asn
290

<210> 33
<211> 527
<212> PRT
<213> tetanolysin-O

<400> 33

Met Asn Lys Asn Val Leu Lys Phe Val Ser Arg Ser Leu Leu Ile Phe
1 5 10 15

Ser Met Thr Gly Leu Ile Ser Asn Tyr Asn Ser Ser Asn Val Leu Ala
20 25 30

Lys Gly Asn Val Glu Glu His Ser Leu Ile Asn Asn Gly Gln Val Val
35 40 45

Thr Ser Asn Thr Lys Cys Asn Leu Ala Lys Asp Asn Ser Ser Asp Ile
50 55 60

Asp Lys Asn Ile Tyr Gly Leu Ser Tyr Asp Pro Arg Lys Ile Leu Ser

65 70 75 80

Tyr Asn Gly Glu Gln Val Glu Asn Phe Val Pro Ala Glu Gly Phe Glu
85 90 95

Asn Pro Asp Lys Phe Ile Val Val Lys Arg Glu Lys Lys Ser Ile Ser
100 105 110

Asp Ser Thr Ala Asp Ile Ser Ile Ile Asp Ser Ile Asn Asp Arg Thr
115 120 125

Tyr Pro Gly Ala Ile Gln Leu Ala Asn Arg Asn Leu Met Glu Asn Lys
130 135 140

Pro Asp Ile Ile Ser Cys Glu Arg Lys Pro Ile Thr Ile Ser Val Asp
145 150 155 160

Leu Pro Gly Met Ala Glu Asp Gly Lys Lys Val Val Asn Ser Pro Thr
165 170 175

Tyr Ser Ser Val Asn Ser Ala Ile Asn Ser Ile Leu Asp Thr Trp Asn
180 185 190

Ser Lys Tyr Ser Ser Lys Tyr Thr Ile Pro Thr Arg Met Ser Tyr Ser
195 200 205

Asp Thr Met Val Tyr Ser Gln Ser Gln Leu Ser Ala Ala Val Gly Cys
210 215 220

Asn Phe Lys Ala Leu Asn Lys Ala Leu Asn Ile Asp Phe Asp Ser Ile
225 230 235 240

Phe Lys Gly Glu Lys Lys Val Met Leu Leu Ala Tyr Lys Gln Ile Phe
245 250 255

Tyr Thr Val Ser Val Asp Pro Pro Asn Arg Pro Ser Asp Leu Phe Gly
260 265 270

Asp Ser Val Thr Phe Asp Glu Leu Ala Leu Lys Gly Ile Asn Asn Asn
275 280 285

Asn Pro Pro Ala Tyr Val Ser Asn Val Ala Tyr Gly Arg Thr Ile Tyr
290 295 300

Val Lys Leu Glu Thr Thr Ser Lys Ser Ser His Val Lys Ala Ala Phe
305 310 315 320

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Lys Ala Leu Ile Asn Asn Gln Asp Ile Ser Ser Asn Ala Glu Tyr Lys
                325                 330                 335

Asp Ile Leu Asn Gln Ser Ser Phe Thr Ala Thr Val Leu Gly Gly Gly
            340                 345                 350

Ala Gln Glu His Asn Lys Ile Ile Thr Lys Asp Phe Asp Glu Ile Arg
        355                 360                 365

Asn Ile Ile Lys Asn Asn Ser Val Tyr Ser Pro Gln Asn Pro Gly Tyr
    370                 375                 380

Pro Ile Ser Tyr Thr Thr Thr Phe Leu Lys Asp Asn Ser Ile Ala Ser
385                 390                 395                 400

Val Asn Asn Lys Thr Glu Tyr Ile Glu Thr Thr Ala Thr Glu Tyr Thr
                405                 410                 415

Asn Gly Lys Ile Val Leu Asp His Ser Gly Ala Tyr Val Ala Gln Phe
            420                 425                 430

Gln Val Thr Trp Asp Glu Val Ser Tyr Asp Glu Lys Gly Asn Glu Ile
        435                 440                 445

Val Glu His Lys Ala Trp Glu Gly Asn Asn Arg Asp Arg Thr Ala His
    450                 455                 460

Phe Asn Thr Glu Ile Tyr Leu Lys Gly Asn Ala Arg Asn Ile Ser Val
465                 470                 475                 480

Lys Ile Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Thr Ile
                485                 490                 495

Val Asp Val Lys Asn Ile Pro Leu Ala Lys Glu Arg Thr Phe Tyr Ile
            500                 505                 510

Trp Gly Thr Thr Leu Tyr Pro Lys Thr Ser Ile Glu Thr Lys Met
        515                 520                 525
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<210> 34
<211> 6129
<212> DNA
<213> Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

<220>
<221> CDS
<222> (133)..(4575)
<223>

<400> 34

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aattggaagc aaatgacatc acagcaggtc agagaaaaag ggttgagcgg caggcaccca      60

gagtagtagg tctttggcat taggagcttg agcccagacg gccctagcag ggaccccagc     120

gcccgagaga cc atg cag agg tcg cct ctg gaa aag gcc agc gtt gtc tcc     171
              Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser
              1               5                   10

aaa ctt ttt ttc agc tgg acc aga cca att ttg agg aaa gga tac aga      219
Lys Leu Phe Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg
    15                  20                  25

cag cgc ctg gaa ttg tca gac ata tac caa atc cct tct gtt gat tct      267
Gln Arg Leu Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser
30                  35                  40                  45

gct gac aat cta tct gaa aaa ttg gaa aga gaa tgg gat aga gag ctg      315
Ala Asp Asn Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu
                50                  55                  60

gct tca aag aaa aat cct aaa ctc att aat gcc ctt cgg cga tgt ttt      363
Ala Ser Lys Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe
            65                  70                  75

ttc tgg aga ttt atg ttc tat gga atc ttt tta tat tta ggg gaa gtc      411
Phe Trp Arg Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val
        80                  85                  90

acc aaa gca gta cag cct ctc tta ctg gga aga atc ata gct tcc tat      459
Thr Lys Ala Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr
    95                  100                 105

gac ccg gat aac aag gag gaa cgc tct atc gcg att tat cta ggc ata      507
Asp Pro Asp Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile
110                 115                 120                 125

ggc tta tgc ctt ctc ttt att gtg agg aca ctg ctc cta cac cca gcc      555
Gly Leu Cys Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala
                130                 135                 140

att ttt ggc ctt cat cac att gga atg cag atg aga ata gct atg ttt      603
Ile Phe Gly Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe
            145                 150                 155

agt ttg att tat aag aag act tta aag ctg tca agc cgt gtt cta gat      651
Ser Leu Ile Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp
        160                 165                 170

aaa ata agt att gga caa ctt gtt agt ctc ctt tcc aac aac ctg aac      699
Lys Ile Ser Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn
    175                 180                 185

aaa ttt gat gaa gga ctt gca ttg gca cat ttc gtg tgg atc gct cct      747
Lys Phe Asp Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro
190                 195                 200                 205

ttg caa gtg gca ctc ctc atg ggg cta atc tgg gag ttg tta cag gcg      795
Leu Gln Val Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala
                210                 215                 220

tct gcc ttc tgt gga ctt ggt ttc ctg ata gtc ctt gcc ctt ttt cag      843
Ser Ala Phe Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln
            225                 230                 235
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gct ggg cta ggg aga atg atg atg aag tac aga gat cag aga gct ggg      891
Ala Gly Leu Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly
        240                 245                 250

aag atc agt gaa aga ctt gtg att acc tca gaa atg att gaa aat atc      939
Lys Ile Ser Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile
    255                 260                 265

caa tct gtt aag gca tac tgc tgg gaa gaa gca atg gaa aaa atg att      987
Gln Ser Val Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile
270                 275                 280                 285

gaa aac tta aga caa aca gaa ctg aaa ctg act cgg aag gca gcc tat     1035
Glu Asn Leu Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr
                290                 295                 300

gtg aga tac ttc aat agc tca gcc ttc ttc tca ggg ttc ttt gtg     1083
Val Arg Tyr Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val
            305                 310                 315

gtg ttt tta tct gtg ctt ccc tat gca cta atc aaa gga atc atc ctc     1131
Val Phe Leu Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu
        320                 325                 330

cgg aaa ata ttc acc acc atc tca ttc tgc att gtt ctg cgc atg gcg     1179
Arg Lys Ile Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala
    335                 340                 345

gtc act cgg caa ttt ccc tgg gct gta caa aca tgg tat gac tct ctt     1227
Val Thr Arg Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu
350                 355                 360                 365

gga gca ata aac aaa ata cag gat ttc tta caa aag caa gaa tat aag     1275
Gly Ala Ile Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys
                370                 375                 380

aca ttg gaa tat aac tta acg act aca gaa gta gtg atg gag aat gta     1323
Thr Leu Glu Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val
            385                 390                 395

aca gcc ttc tgg gag gag gga ttt ggg gaa tta ttt gag aaa gca aaa     1371
Thr Ala Phe Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys
        400                 405                 410

caa aac aat aac aat aga aaa act tct aat ggt gat gac agc ctc ttc     1419
Gln Asn Asn Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe
    415                 420                 425

ttc agt aat ttc tca ctt ctt ggt act cct gtc ctg aaa gat att aat     1467
Phe Ser Asn Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn
430                 435                 440                 445

ttc aag ata gaa aga gga cag ttg ttg gcg gtt gct gga tcc act gga     1515
Phe Lys Ile Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly
                450                 455                 460

gca ggc aag act tca ctt cta atg atg att atg gga gaa ctg gag cct     1563
Ala Gly Lys Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro
            465                 470                 475

tca gag ggt aaa att aag cac agt gga aga att tca ttc tgt tct cag     1611
Ser Glu Gly Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln
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        480                 485                 490

ttt tcc tgg att atg cct ggc acc att aaa gaa aat atc atc ttt ggt     1659
Phe Ser Trp Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly
    495                 500                 505

gtt tcc tat gat gaa tat aga tac aga agc gtc atc aaa gca tgc caa     1707
Val Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln
510                 515                 520                 525

cta gaa gag gac atc tcc aag ttt gca gag aaa gac aat ata gtt ctt     1755
Leu Glu Glu Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu
                530                 535                 540

gga gaa ggt gga atc aca ctg agt gga ggt caa cga gca aga att tct     1803
Gly Glu Gly Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser
            545                 550                 555

tta gca aga gca gta tac aaa gat gct gat ttg tat tta tta gac tct     1851
Leu Ala Arg Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser
        560                 565                 570

cct ttt gga tac cta gat gtt tta aca gaa aaa gaa ata ttt gaa agc     1899
Pro Phe Gly Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser
    575                 580                 585

tgt gtc tgt aaa ctg atg gct aac aaa act agg att ttg gtc act tct     1947
Cys Val Cys Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser
590                 595                 600                 605

aaa atg gaa cat tta aag aaa gct gac aaa ata tta att ttg aat gaa     1995
Lys Met Glu His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu
                610                 615                 620

ggt agc agc tat ttt tat ggg aca ttt tca gaa ctc caa aat cta cag     2043
Gly Ser Ser Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln
            625                 630                 635

cca gac ttt agc tca aaa ctc atg gga tgt gat tct ttc gac caa ttt     2091
Pro Asp Phe Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe
        640                 645                 650

agt gca gaa aga aga aat tca atc cta act gag acc tta cac cgt ttc     2139
Ser Ala Glu Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe
    655                 660                 665

tca tta gaa gga gat gct cct gtc tcc tgg aca gaa aca aaa aaa caa     2187
Ser Leu Glu Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln
670                 675                 680                 685

tct ttt aaa cag act gga gag ttt ggg gaa aaa agg aag aat tct att     2235
Ser Phe Lys Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile
                690                 695                 700

ctc aat cca atc aac tct ata cga aaa ttt tcc att gtg caa aag act     2283
Leu Asn Pro Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr
            705                 710                 715

ccc tta caa atg aat ggc atc gaa gag gat tct gat gag cct tta gag     2331
Pro Leu Gln Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu
        720                 725                 730

aga agg ctg tcc tta gta cca gat tct gag cag gga gag gcg ata ctg     2379
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Arg Arg Leu Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu
    735                 740                 745

cct cgc atc agc gtg atc agc act ggc ccc acg ctt cag gca cga agg     2427
Pro Arg Ile Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg
750                 755                 760                 765

agg cag tct gtc ctg aac ctg atg aca cac tca gtt aac caa ggt cag     2475
Arg Gln Ser Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln
                770                 775                 780

aac att cac cga aag aca aca gca tcc aca cga aaa gtg tca ctg gcc     2523
Asn Ile His Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala
            785                 790                 795

cct cag gca aac ttg act gaa ctg gat ata tat tca aga agg tta tct     2571
Pro Gln Ala Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser
        800                 805                 810

caa gaa act ggc ttg gaa ata agt gaa gaa att aac gaa gaa gac tta     2619
Gln Glu Thr Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu
    815                 820                 825

aag gag tgc ctt ttt gat gat atg gag agc ata cca gca gtg act aca     2667
Lys Glu Cys Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr
830                 835                 840                 845

tgg aac aca tac ctt cga tat att act gtc cac aag agc tta att ttt     2715
Trp Asn Thr Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe
                850                 855                 860

gtg cta att tgg tgc tta gta att ttt ctg gca gag gtg gct gct tct     2763
Val Leu Ile Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser
            865                 870                 875

ttg gtt gtg ctg tgg ctc ctt gga aac act cct ctt caa gac aaa ggg     2811
Leu Val Val Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly
        880                 885                 890

aat agt act cat agt aga aat aac agc tat gca gtg att atc acc agc     2859
Asn Ser Thr His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser
    895                 900                 905

acc agt tcg tat tat gtg ttt tac att tac gtg gga gta gcc gac act     2907
Thr Ser Ser Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr
910                 915                 920                 925

ttg ctt gct atg gga ttc ttc aga ggt cta cca ctg gtg cat act cta     2955
Leu Leu Ala Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu
                930                 935                 940

atc aca gtg tcg aaa att tta cac cac aaa atg tta cat tct gtt ctt     3003
Ile Thr Val Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu
            945                 950                 955

caa gca cct atg tca acc ctc aac acg ttg aaa gca ggt ggg att ctt     3051
Gln Ala Pro Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu
        960                 965                 970

aat aga ttc tcc aaa gat ata gca att ttg gat gac ctt ctg cct ctt     3099
Asn Arg Phe Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu
    975                 980                 985
```

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acc ata ttt gac ttc atc cag ttg tta tta att  gtg att gga gct ata    3147
Thr Ile Phe Asp Phe Ile Gln Leu Leu Leu Ile  Val Ile Gly Ala Ile
990                 995                 1000                1005

gca gtt gtc gca gtt  tta caa ccc tac atc  ttt gtt gca aca gtg       3192
Ala Val Val Ala Val  Leu Gln Pro Tyr Ile  Phe Val Ala Thr Val
                1010                1015                1020

cca gtg ata gtg gct  ttt att atg ttg aga  gca tat ttc ctc caa       3237
Pro Val Ile Val Ala  Phe Ile Met Leu Arg  Ala Tyr Phe Leu Gln
                1025                1030                1035

acc tca cag caa ctc  aaa caa ctg gaa tct  gaa ggc agg agt cca       3282
Thr Ser Gln Gln Leu  Lys Gln Leu Glu Ser  Glu Gly Arg Ser Pro
                1040                1045                1050

att ttc act cat ctt  gtt aca agc tta aaa  gga cta tgg aca ctt       3327
Ile Phe Thr His Leu  Val Thr Ser Leu Lys  Gly Leu Trp Thr Leu
                1055                1060                1065

cgt gcc ttc gga cgg  cag cct tac ttt gaa  act ctg ttc cac aaa       3372
Arg Ala Phe Gly Arg  Gln Pro Tyr Phe Glu  Thr Leu Phe His Lys
                1070                1075                1080

gct ctg aat tta cat  act gcc aac tgg ttc  ttg tac ctg tca aca       3417
Ala Leu Asn Leu His  Thr Ala Asn Trp Phe  Leu Tyr Leu Ser Thr
                1085                1090                1095

ctg cgc tgg ttc caa  atg aga ata gaa atg  att ttt gtc atc ttc       3462
Leu Arg Trp Phe Gln  Met Arg Ile Glu Met  Ile Phe Val Ile Phe
                1100                1105                1110

ttc att gct gtt acc  ttc att tcc att tta  aca aca gga gaa gga       3507
Phe Ile Ala Val Thr  Phe Ile Ser Ile Leu  Thr Thr Gly Glu Gly
                1115                1120                1125

gaa gga aga gtt ggt  att atc ctg act tta  gcc atg aat atc atg       3552
Glu Gly Arg Val Gly  Ile Ile Leu Thr Leu  Ala Met Asn Ile Met
                1130                1135                1140

agt aca ttg cag tgg  gct gta aac tcc agc  ata gat gtg gat agc       3597
Ser Thr Leu Gln Trp  Ala Val Asn Ser Ser  Ile Asp Val Asp Ser
                1145                1150                1155

ttg atg cga tct gtg  agc cga gtc ttt aag  ttc att gac atg cca       3642
Leu Met Arg Ser Val  Ser Arg Val Phe Lys  Phe Ile Asp Met Pro
                1160                1165                1170

aca gaa ggt aaa cct  acc aag tca acc aaa  cca tac aag aat ggc       3687
Thr Glu Gly Lys Pro  Thr Lys Ser Thr Lys  Pro Tyr Lys Asn Gly
                1175                1180                1185

caa ctc tcg aaa gtt  atg att att gag aat  tca cac gtg aag aaa       3732
Gln Leu Ser Lys Val  Met Ile Ile Glu Asn  Ser His Val Lys Lys
                1190                1195                1200

gat gac atc tgg ccc  tca ggg ggc caa atg  act gtc aaa gat ctc       3777
Asp Asp Ile Trp Pro  Ser Gly Gly Gln Met  Thr Val Lys Asp Leu
                1205                1210                1215

aca gca aaa tac aca  gaa ggt gga aat gcc  ata tta gag aac att       3822
Thr Ala Lys Tyr Thr  Glu Gly Gly Asn Ala  Ile Leu Glu Asn Ile
                1220                1225                1230
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tcc ttc tca ata agt  cct ggc cag agg gtg  ggc ctc ttg gga aga       3867
Ser Phe Ser Ile Ser  Pro Gly Gln Arg Val  Gly Leu Leu Gly Arg
                1235                1240                1245

act gga tca ggg aag  agt act ttg tta tca  gct ttt ttg aga cta       3912
Thr Gly Ser Gly Lys  Ser Thr Leu Leu Ser  Ala Phe Leu Arg Leu
                1250                1255                1260

ctg aac act gaa gga  gaa atc cag atc gat  ggt gtg tct tgg gat       3957
Leu Asn Thr Glu Gly  Glu Ile Gln Ile Asp  Gly Val Ser Trp Asp
                1265                1270                1275

tca ata act ttg caa  cag tgg agg aaa gcc  ttt gga gtg ata cca       4002
Ser Ile Thr Leu Gln  Gln Trp Arg Lys Ala  Phe Gly Val Ile Pro
                1280                1285                1290

cag aaa gta ttt att  ttt tct gga aca ttt  aga aaa aac ttg gat       4047
Gln Lys Val Phe Ile  Phe Ser Gly Thr Phe  Arg Lys Asn Leu Asp
                1295                1300                1305

ccc tat gaa cag tgg  agt gat caa gaa ata  tgg aaa gtt gca gat       4092
Pro Tyr Glu Gln Trp  Ser Asp Gln Glu Ile  Trp Lys Val Ala Asp
                1310                1315                1320

gag gtt ggg ctc aga  tct gtg ata gaa cag  ttt cct ggg aag ctt       4137
Glu Val Gly Leu Arg  Ser Val Ile Glu Gln  Phe Pro Gly Lys Leu
                1325                1330                1335

gac ttt gtc ctt gtg  gat ggg ggc tgt gtc  cta agc cat ggc cac       4182
Asp Phe Val Leu Val  Asp Gly Gly Cys Val  Leu Ser His Gly His
                1340                1345                1350

aag cag ttg atg tgc  ttg gct aga tct gtt  ctc agt aag gcg aag       4227
Lys Gln Leu Met Cys  Leu Ala Arg Ser Val  Leu Ser Lys Ala Lys
                1355                1360                1365

atc ttg ctg ctt gat  gaa ccc agt gct cat  ttg gat cca gta aca       4272
Ile Leu Leu Leu Asp  Glu Pro Ser Ala His  Leu Asp Pro Val Thr
                1370                1375                1380

tac caa ata att aga  aga act cta aaa caa  gca ttt gct gat tgc       4317
Tyr Gln Ile Ile Arg  Arg Thr Leu Lys Gln  Ala Phe Ala Asp Cys
                1385                1390                1395

aca gta att ctc tgt  gaa cac agg ata gaa  gca atg ctg gaa tgc       4362
Thr Val Ile Leu Cys  Glu His Arg Ile Glu  Ala Met Leu Glu Cys
                1400                1405                1410

caa caa ttt ttg gtc  ata gaa gag aac aaa  gtg cgg cag tac gat       4407
Gln Gln Phe Leu Val  Ile Glu Glu Asn Lys  Val Arg Gln Tyr Asp
                1415                1420                1425

tcc atc cag aaa ctg  ctg aac gag agg agc  ctc ttc cgg caa gcc       4452
Ser Ile Gln Lys Leu  Leu Asn Glu Arg Ser  Leu Phe Arg Gln Ala
                1430                1435                1440

atc agc ccc tcc gac  agg gtg aag ctc ttt  ccc cac cgg aac tca       4497
Ile Ser Pro Ser Asp  Arg Val Lys Leu Phe  Pro His Arg Asn Ser
                1445                1450                1455

agc aag tgc aag tct  aag ccc cag att gct  gct ctg aaa gag gag       4542
Ser Lys Cys Lys Ser  Lys Pro Gln Ile Ala  Ala Leu Lys Glu Glu
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                1460                1465                1470

aca gaa gaa gag gtg  caa gat aca agg ctt  tag agagcagcat            4585
Thr Glu Glu Glu Val  Gln Asp Thr Arg Leu
                1475                1480

aaatgttgac atgggacatt tgctcatgga attggagctc gtgggacagt cacctcatgg   4645

aattggagct cgtggaacag ttacctctgc ctcagaaaac aaggatgaat taagtttttt   4705

tttaaaaaag aaacatttgg taaggggaat tgaggacact gatatgggtc ttgataaatg   4765

gcttcctggc aatagtcaaa ttgtgtgaaa ggtacttcaa atccttgaag atttaccact   4825

tgtgttttgc aagccagatt ttcctgaaaa cccttgccat gtgctagtaa ttggaaaggc   4885

agctctaaat gtcaatcagc ctagttgatc agcttattgt ctagtgaaac tcgttaattt   4945

gtagtgttgg agaagaactg aaatcatact tcttagggtt atgattaagt aatgataact   5005

ggaaacttca gcggtttata taagcttgta ttccttttc tctcctctcc ccatgatgtt   5065

tagaaacaca actatattgt ttgctaagca ttccaactat ctcatttcca agcaagtatt   5125

agaataccac aggaaccaca agactgcaca tcaaaatatg ccccattcaa catctagtga   5185

gcagtcagga aagagaactt ccagatcctg gaaatcaggg ttagtattgt ccaggtctac   5245

caaaaatctc aatatttcag ataatcacaa tacatccctt acctgggaaa gggctgttat   5305

aatctttcac aggggacagg atggttccct tgatgaagaa gttgatatgc cttttcccaa   5365

ctccagaaag tgacaagctc acagaccttt gaactagagt ttagctggaa aagtatgtta   5425

gtgcaaattg tcacaggaca gcccttcttt ccacagaagc tccaggtaga gggtgtgtaa   5485

gtagataggc catgggcact gtgggtagac acacatgaag tccaagcatt tagatgtata   5545

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agaatgagag acacactgaa gaagcaccaa tcatgaatta gttttatatg cttctgtttt   5665

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atatattaca atgctgtatt ttaaaagaat gattatgaat tacatttgta taaaataatt   5785

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cacagcctct tagatgcagt tctgaagaag atggtaccac cagtctgact gtttccatca   6025

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Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg Gln Arg Leu
20 25 30

Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser Ala Asp Asn
35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
115 120 125

Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala Ile Phe Gly
130 135 140

Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
210 215 220

Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
225 230 235 240

```
Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly Lys Ile Ser
               245                 250                 255

Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile Gln Ser Val
           260                 265                 270

Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
       275                 280                 285

Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
   290                 295                 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305                 310                 315                 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
               325                 330                 335

Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
           340                 345                 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
       355                 360                 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
   370                 375                 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
385                 390                 395                 400

Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
               405                 410                 415

Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe Phe Ser Asn
           420                 425                 430

Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
       435                 440                 445

Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly Ala Gly Lys
   450                 455                 460

Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro Ser Glu Gly
465                 470                 475                 480

Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln Phe Ser Trp
               485                 490                 495
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Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Phe Gly Val Ser Tyr
500 505 510

Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu
515 520 525

Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly Glu Gly
530 535 540

Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu Ala Arg
545 550 555 560

Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly
565 570 575

Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys Val Cys
580 585 590

Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys Met Glu
595 600 605

His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly Ser Ser
610 615 620

Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro Asp Phe
625 630 635 640

Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser Ala Glu
645 650 655

Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu
660 665 670

Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys
675 680 685

Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro
690 695 700

Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln
705 710 715 720

Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu
725 730 735

Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro Arg Ile
740 745 750

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Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser
        755                 760                 765

Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn Ile His
    770                 775                 780

Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro Gln Ala
785                 790                 795                 800

Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln Glu Thr
                805                 810                 815

Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys Glu Cys
            820                 825                 830

Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp Asn Thr
        835                 840                 845

Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile
    850                 855                 860

Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu Val Val
865                 870                 875                 880

Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn Ser Thr
                885                 890                 895

His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr Ser Ser
            900                 905                 910

Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala
        915                 920                 925

Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val
    930                 935                 940

Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro
945                 950                 955                 960

Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe
                965                 970                 975

Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr Ile Phe
            980                 985                 990

Asp Phe Ile Gln Leu Leu Leu Ile  Val Ile Gly Ala Ile  Ala Val Val
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995 1000 1005

Ala Val Leu Gln Pro Tyr Ile Phe Val Ala Thr Val Pro Val Ile
1010 1015 1020

Val Ala Phe Ile Met Leu Arg Ala Tyr Phe Leu Gln Thr Ser Gln
1025 1030 1035

Gln Leu Lys Gln Leu Glu Ser Glu Gly Arg Ser Pro Ile Phe Thr
1040 1045 1050

His Leu Val Thr Ser Leu Lys Gly Leu Trp Thr Leu Arg Ala Phe
1055 1060 1065

Gly Arg Gln Pro Tyr Phe Glu Thr Leu Phe His Lys Ala Leu Asn
1070 1075 1080

Leu His Thr Ala Asn Trp Phe Leu Tyr Leu Ser Thr Leu Arg Trp
1085 1090 1095

Phe Gln Met Arg Ile Glu Met Ile Phe Val Ile Phe Phe Ile Ala
1100 1105 1110

Val Thr Phe Ile Ser Ile Leu Thr Thr Gly Glu Gly Glu Gly Arg
1115 1120 1125

Val Gly Ile Ile Leu Thr Leu Ala Met Asn Ile Met Ser Thr Leu
1130 1135 1140

Gln Trp Ala Val Asn Ser Ser Ile Asp Val Asp Ser Leu Met Arg
1145 1150 1155

Ser Val Ser Arg Val Phe Lys Phe Ile Asp Met Pro Thr Glu Gly
1160 1165 1170

Lys Pro Thr Lys Ser Thr Lys Pro Tyr Lys Asn Gly Gln Leu Ser
1175 1180 1185

Lys Val Met Ile Ile Glu Asn Ser His Val Lys Lys Asp Asp Ile
1190 1195 1200

Trp Pro Ser Gly Gly Gln Met Thr Val Lys Asp Leu Thr Ala Lys
1205 1210 1215

Tyr Thr Glu Gly Gly Asn Ala Ile Leu Glu Asn Ile Ser Phe Ser
1220 1225 1230

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Ile Ser  Pro Gly Gln Arg Val  Gly Leu Leu Gly Arg  Thr Gly Ser
    1235                 1240                 1245

Gly Lys  Ser Thr Leu Leu Ser  Ala Phe Leu Arg Leu  Leu Asn Thr
    1250                 1255                 1260

Glu Gly  Glu Ile Gln Ile Asp  Gly Val Ser Trp Asp  Ser Ile Thr
    1265                 1270                 1275

Leu Gln  Gln Trp Arg Lys Ala  Phe Gly Val Ile Pro  Gln Lys Val
    1280                 1285                 1290

Phe Ile  Phe Ser Gly Thr Phe  Arg Lys Asn Leu Asp  Pro Tyr Glu
    1295                 1300                 1305

Gln Trp  Ser Asp Gln Glu Ile  Trp Lys Val Ala Asp  Glu Val Gly
    1310                 1315                 1320

Leu Arg  Ser Val Ile Glu Gln  Phe Pro Gly Lys Leu  Asp Phe Val
    1325                 1330                 1335

Leu Val  Asp Gly Gly Cys Val  Leu Ser His Gly His  Lys Gln Leu
    1340                 1345                 1350

Met Cys  Leu Ala Arg Ser Val  Leu Ser Lys Ala Lys  Ile Leu Leu
    1355                 1360                 1365

Leu Asp  Glu Pro Ser Ala His  Leu Asp Pro Val Thr  Tyr Gln Ile
    1370                 1375                 1380

Ile Arg  Arg Thr Leu Lys Gln  Ala Phe Ala Asp Cys  Thr Val Ile
    1385                 1390                 1395

Leu Cys  Glu His Arg Ile Glu  Ala Met Leu Glu Cys  Gln Gln Phe
    1400                 1405                 1410

Leu Val  Ile Glu Glu Asn Lys  Val Arg Gln Tyr Asp  Ser Ile Gln
    1415                 1420                 1425

Lys Leu  Leu Asn Glu Arg Ser  Leu Phe Arg Gln Ala  Ile Ser Pro
    1430                 1435                 1440

Ser Asp  Arg Val Lys Leu Phe  Pro His Arg Asn Ser  Ser Lys Cys
    1445                 1450                 1455

Lys Ser  Lys Pro Gln Ile Ala  Ala Leu Lys Glu Glu  Thr Glu Glu
    1460                 1465                 1470
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Glu Val  Gln Asp Thr Arg Leu
    1475                1480


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Thr Gly Thr Leu Val Phe Thr Val Ile Thr Ala Val Leu Gly Ser Phe
            10                  15                  20

cag ttt gga tat gac att ggt gtg atc aat gca cct caa cag gta ata     152
Gln Phe Gly Tyr Asp Ile Gly Val Ile Asn Ala Pro Gln Gln Val Ile
        25                  30                  35

ata tct cac tat aga cat gtt ttg ggt gtt cca ctg gat gac cga aaa     200
Ile Ser His Tyr Arg His Val Leu Gly Val Pro Leu Asp Asp Arg Lys
    40                  45                  50

gct atc aac aac tat gtt atc aac agt aca gat gaa ctg ccc aca atc     248
Ala Ile Asn Asn Tyr Val Ile Asn Ser Thr Asp Glu Leu Pro Thr Ile
55                  60                  65                  70

tca tac tca atg aac cca aaa cca acc cct tgg gct gag gaa gag act     296
Ser Tyr Ser Met Asn Pro Lys Pro Thr Pro Trp Ala Glu Glu Glu Thr
                75                  80                  85

gtg gca gct gct caa cta atc acc atg ctc tgg tcc ctg tct gta tcc     344
Val Ala Ala Ala Gln Leu Ile Thr Met Leu Trp Ser Leu Ser Val Ser
            90                  95                  100

agc ttt gca gtt ggt gga atg act gca tca ttc ttt ggt ggg tgg ctt     392
Ser Phe Ala Val Gly Gly Met Thr Ala Ser Phe Phe Gly Gly Trp Leu
        105                 110                 115

ggg gac aca ctt gga aga atc aaa gcc atg tta gta gca aac att ctg     440
Gly Asp Thr Leu Gly Arg Ile Lys Ala Met Leu Val Ala Asn Ile Leu
    120                 125                 130

tca tta gtt gga gct ctc ttg atg ggg ttt tca aaa ttg gga cca tct     488
Ser Leu Val Gly Ala Leu Leu Met Gly Phe Ser Lys Leu Gly Pro Ser
135                 140                 145                 150

cat ata ctt ata att gct gga aga agc ata tca gga cta tat tgt ggg     536
His Ile Leu Ile Ile Ala Gly Arg Ser Ile Ser Gly Leu Tyr Cys Gly
                155                 160                 165

cta att tca ggc ctg gtt cct atg tat atc ggt gaa att gct cca acc     584
Leu Ile Ser Gly Leu Val Pro Met Tyr Ile Gly Glu Ile Ala Pro Thr
            170                 175                 180
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gct ctc agg gga gca ctt ggc act ttt cat cag ctg gcc atc gtc acg      632
Ala Leu Arg Gly Ala Leu Gly Thr Phe His Gln Leu Ala Ile Val Thr
        185                 190                 195

ggc att ctt att agt cag att att ggt ctt gaa ttt atc ttg ggc aat      680
Gly Ile Leu Ile Ser Gln Ile Ile Gly Leu Glu Phe Ile Leu Gly Asn
    200                 205                 210

tat gat ctg tgg cac atc ctg ctt ggc ctg tct ggt gtg cga gcc atc      728
Tyr Asp Leu Trp His Ile Leu Leu Gly Leu Ser Gly Val Arg Ala Ile
215                 220                 225                 230

ctt cag tct ctg cta ctc ttt ttc tgt cca gaa agc ccc aga tac ctt      776
Leu Gln Ser Leu Leu Leu Phe Phe Cys Pro Glu Ser Pro Arg Tyr Leu
                235                 240                 245

tac atc aag tta gat gag gaa gtc aaa gca aaa caa agc ttg aaa aga      824
Tyr Ile Lys Leu Asp Glu Glu Val Lys Ala Lys Gln Ser Leu Lys Arg
            250                 255                 260

ctc aga gga tat gat gat gtc acc aaa gat att aat gaa atg aga aaa      872
Leu Arg Gly Tyr Asp Asp Val Thr Lys Asp Ile Asn Glu Met Arg Lys
        265                 270                 275

gaa aga gaa gaa gca tcg agt gag cag aaa gtc tct ata att cag ctc      920
Glu Arg Glu Glu Ala Ser Ser Glu Gln Lys Val Ser Ile Ile Gln Leu
    280                 285                 290

ttc acc aat tcc agc tac cga cag cct att cta gtg gca ctg atg ctg      968
Phe Thr Asn Ser Ser Tyr Arg Gln Pro Ile Leu Val Ala Leu Met Leu
295                 300                 305                 310

cat gtg gct cag caa ttt tcc gga atc aat ggc att ttt tac tac tca     1016
His Val Ala Gln Gln Phe Ser Gly Ile Asn Gly Ile Phe Tyr Tyr Ser
                315                 320                 325

acc agc att ttt cag acg gct ggt atc agc aaa cct gtt tat gca acc     1064
Thr Ser Ile Phe Gln Thr Ala Gly Ile Ser Lys Pro Val Tyr Ala Thr
            330                 335                 340

att gga gtt ggc gct gta aac atg gtt ttc act gct gtc tct gta ttc     1112
Ile Gly Val Gly Ala Val Asn Met Val Phe Thr Ala Val Ser Val Phe
        345                 350                 355

ctt gtg gag aag gca ggg cga cgt tct ctc ttt cta att gga atg agt     1160
Leu Val Glu Lys Ala Gly Arg Arg Ser Leu Phe Leu Ile Gly Met Ser
    360                 365                 370

ggg atg ttt gtt tgt gcc atc ttc atg tca gtg gga ctt gtg ctg ctg     1208
Gly Met Phe Val Cys Ala Ile Phe Met Ser Val Gly Leu Val Leu Leu
375                 380                 385                 390

aat aag ttc tct tgg atg agt tat gtg agc atg ata gcc atc ttc ctc     1256
Asn Lys Phe Ser Trp Met Ser Tyr Val Ser Met Ile Ala Ile Phe Leu
                395                 400                 405

ttt gtc agc ttc ttt gaa att ggg cca ggc ccg atc ccc tgg ttc atg     1304
Phe Val Ser Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp Phe Met
            410                 415                 420

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Ala Ala Phe Ser Asn Trp Thr Cys Asn Phe Ile Val Ala Leu Cys Phe
    440                 445                 450

cag tac att gcg gac ttc tgt gga cct tat gtg ttt ttc ctc ttt gct     1448
Gln Tyr Ile Ala Asp Phe Cys Gly Pro Tyr Val Phe Phe Leu Phe Ala
455                 460                 465                 470

gga gtg ctc ctg gcc ttt acc ctg ttc aca ttt ttt aaa gtt cca gaa     1496
Gly Val Leu Leu Ala Phe Thr Leu Phe Thr Phe Phe Lys Val Pro Glu
                475                 480                 485

acc aaa gga aag tct ttt gag gaa att gct gca gaa ttc caa aag aag     1544
Thr Lys Gly Lys Ser Phe Glu Glu Ile Ala Ala Glu Phe Gln Lys Lys
            490                 495                 500

agt ggc tca gcc cac agg cca aaa gct gct gta gaa atg aaa ttc cta     1592
Ser Gly Ser Ala His Arg Pro Lys Ala Ala Val Glu Met Lys Phe Leu
        505                 510                 515

gga gct aca gag act gtg taa aaaaaaaacc ctgctttttg acatgaacag       1643
Gly Ala Thr Glu Thr Val
    520

aaacaataag ggaaccgtct gtttttaaat gatgattcct tgagcatttt atatccacat   1703

ctttaagtat tgttttattt ttatgtgctc tcatcagaaa tgtcatcaaa tattaccaaa   1763

aaagtatttt tttaagttag agaatatatt tttgatggta agactgtaat taagtaaacc   1823

aaaaaggcta gtttatttttg ttacactaaa gggcaggtgg ttctaatatt tttagctctg   1883

ttctttataa caaggttctt ctaaaattga agagatttca acatatcatt tttttaacac   1943

ataactagaa acctgaggat gcaacaaata tttatatatt tgaatatcat taaattggaa   2003

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ctaaaaaatg tctatagaga aaaactttca tttttggtag gcttatcaaa atctttcagc   2243

actcagaaaa gaaaaccatt ttagttcctt tatttaatgg ccaaatggtt tttgcaagat   2303

ttaacactaa aaaggtttca cctgatcata tagcgtgggt tatcagttaa cattaacatc   2363

tattataaaa ccatgttgat tcccttctgg tacaatcctt tgagttatag tttgctttgc   2423

tttttaattg aggacagcct ggttttcaca tacactcaaa caatcatgag tcagacattt   2483

ggtatattac ctcaaattcc taataagttt gatcaaatct aatgtaagaa aatttgaagt   2543

aaaggattga tcactttgtt aaaaatattt tctgaattat tatgtctcaa aataagttga   2603

aaaggtaggg tttgaggatt cctgagtgtg ggcttctgaa acttcataaa tgttcagctt   2663

cagactttta tcaaaatccc tatttaattt tcctggaaag actgattgtt ttatggtgtg   2723

ttcctaacat aaaataatcg tctcctttga catttccttc tttgtcttag ctgtatacag   2783
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attctagcca aactattcta tggccattac taacacgcat tgtacactat ctatctgcct 2843

ttacctacat aggcaaattg gaaatacaca gatgattaaa cagactttag cttacagtca 2903

attttacaat tatggaaata tagttctgat gggtcccaaa agcttagcag ggtgctaacg 2963

tatctctagg ctgttttctc caccaactgg agcactgatc aatccttctt atgtttgctt 3023

taatgtgtat tgaagaaaag cacttttttaa aaagtactct ttaagagtga aataattaaa 3083

aaccactgaa catttgcttt gttttctaaa gttgttcaca tatatgtaat ttagcagtcc 3143

aaagaacaag aaattgtttc ttttc 3168

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<400> 37

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Ala Val Leu Gly Ser Phe Gln Phe Gly Tyr Asp Ile Gly Val Ile Asn
20 25 30

Ala Pro Gln Gln Val Ile Ile Ser His Tyr Arg His Val Leu Gly Val
35 40 45

Pro Leu Asp Asp Arg Lys Ala Ile Asn Asn Tyr Val Ile Asn Ser Thr
50 55 60

Asp Glu Leu Pro Thr Ile Ser Tyr Ser Met Asn Pro Lys Pro Thr Pro
65 70 75 80

Trp Ala Glu Glu Glu Thr Val Ala Ala Ala Gln Leu Ile Thr Met Leu
85 90 95

Trp Ser Leu Ser Val Ser Ser Phe Ala Val Gly Gly Met Thr Ala Ser
100 105 110

Phe Phe Gly Gly Trp Leu Gly Asp Thr Leu Gly Arg Ile Lys Ala Met
115 120 125

Leu Val Ala Asn Ile Leu Ser Leu Val Gly Ala Leu Leu Met Gly Phe
130 135 140

Ser Lys Leu Gly Pro Ser His Ile Leu Ile Ile Ala Gly Arg Ser Ile
145 150 155 160

Ser Gly Leu Tyr Cys Gly Leu Ile Ser Gly Leu Val Pro Met Tyr Ile
165 170 175

Gly Glu Ile Ala Pro Thr Ala Leu Arg Gly Ala Leu Gly Thr Phe His
180 185 190

Gln Leu Ala Ile Val Thr Gly Ile Leu Ile Ser Gln Ile Ile Gly Leu
195 200 205

Glu Phe Ile Leu Gly Asn Tyr Asp Leu Trp His Ile Leu Leu Gly Leu
210 215 220

Ser Gly Val Arg Ala Ile Leu Gln Ser Leu Leu Leu Phe Phe Cys Pro
225 230 235 240

Glu Ser Pro Arg Tyr Leu Tyr Ile Lys Leu Asp Glu Glu Val Lys Ala
245 250 255

Lys Gln Ser Leu Lys Arg Leu Arg Gly Tyr Asp Asp Val Thr Lys Asp
260 265 270

Ile Asn Glu Met Arg Lys Glu Arg Glu Glu Ala Ser Ser Glu Gln Lys
275 280 285

Val Ser Ile Ile Gln Leu Phe Thr Asn Ser Ser Tyr Arg Gln Pro Ile
290 295 300

Leu Val Ala Leu Met Leu His Val Ala Gln Gln Phe Ser Gly Ile Asn
305 310 315 320

Gly Ile Phe Tyr Tyr Ser Thr Ser Ile Phe Gln Thr Ala Gly Ile Ser
325 330 335

Lys Pro Val Tyr Ala Thr Ile Gly Val Gly Ala Val Asn Met Val Phe
340 345 350

Thr Ala Val Ser Val Phe Leu Val Glu Lys Ala Gly Arg Arg Ser Leu
355 360 365

Phe Leu Ile Gly Met Ser Gly Met Phe Val Cys Ala Ile Phe Met Ser
370 375 380

Val Gly Leu Val Leu Leu Asn Lys Phe Ser Trp Met Ser Tyr Val Ser
385 390 395 400

Met Ile Ala Ile Phe Leu Phe Val Ser Phe Phe Glu Ile Gly Pro Gly
405 410 415

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Pro Ile Pro Trp Phe Met Val Ala Glu Phe Phe Ser Gln Gly Pro Arg
            420                 425                 430

Pro Ala Ala Leu Ala Ile Ala Ala Phe Ser Asn Trp Thr Cys Asn Phe
        435                 440                 445

Ile Val Ala Leu Cys Phe Gln Tyr Ile Ala Asp Phe Cys Gly Pro Tyr
    450                 455                 460

Val Phe Phe Leu Phe Ala Gly Val Leu Leu Ala Phe Thr Leu Phe Thr
465                 470                 475                 480

Phe Phe Lys Val Pro Glu Thr Lys Gly Lys Ser Phe Glu Glu Ile Ala
            485                 490                 495

Ala Glu Phe Gln Lys Lys Ser Gly Ser Ala His Arg Pro Lys Ala Ala
            500                 505                 510

Val Glu Met Lys Phe Leu Gly Ala Thr Glu Thr Val
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ttcctgagga cgtggagaaa actttctgct gagaaggaca ttttgaaggt tttgttggct    180

gaaaaagctg tttctggaat cacccctaga tctttcttga agacttgaat tagattacag    240

cg atg ggg aca cag aag gtc acc cca gct ctg ata ttt gcc atc aca       287
   Met Gly Thr Gln Lys Val Thr Pro Ala Leu Ile Phe Ala Ile Thr
   1               5                  10                  15

gtt gct aca atc ggc tct ttc caa ttt ggc tac aac act ggg gtc atc      335
Val Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile
                20                  25                  30

aat gct cct gag aag atc ata aag gaa ttt atc aat aaa act ttg acg      383
Asn Ala Pro Glu Lys Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr
            35                  40                  45

gac aag gga aat gcc cca ccc tct gag gtg ctg ctc acg tct ctc tgg      431
Asp Lys Gly Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Ser Leu Trp
        50                  55                  60
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tcc ttg tct gtg gcc ata ttt tcc gtc ggg ggt atg atc ggc tcc ttt      479
Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe
    65                  70                  75

tcc gtc gga ctc ttc gtc aac cgc ttt ggc agg cgc aat tca atg ctg      527
Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu
80                  85                  90                  95

att gtc aac ctg ttg gct gtc act ggt ggc tgc ttt atg gga ctg tgt      575
Ile Val Asn Leu Leu Ala Val Thr Gly Gly Cys Phe Met Gly Leu Cys
                100                 105                 110

aaa gta gct aag tcg gtt gaa atg ctg atc ctg ggt cgc ttg gtt att      623
Lys Val Ala Lys Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile
            115                 120                 125

ggc ctc ttc tgc gga ctc tgc aca ggt ttt gtg ccc atg tac att gga      671
Gly Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly
        130                 135                 140

gag atc tcg cct act gcc ctg cgg ggt gcc ttt ggc act ctc aac cag      719
Glu Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln
    145                 150                 155

ctg ggc atc gtt gtt gga att ctg gtg gcc cag atc ttt ggt ctg gaa      767
Leu Gly Ile Val Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu
160                 165                 170                 175

ttc atc ctt ggg tct gaa gag cta tgg ccg ctg cta ctg ggt ttt acc      815
Phe Ile Leu Gly Ser Glu Glu Leu Trp Pro Leu Leu Leu Gly Phe Thr
                180                 185                 190

atc ctt cct gct atc cta caa agt gca gcc ctt cca ttt tgc cct gaa      863
Ile Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Phe Cys Pro Glu
            195                 200                 205

agt ccc aga ttt ttg ctc att aac aga aaa gaa gag gag aat gct aag      911
Ser Pro Arg Phe Leu Leu Ile Asn Arg Lys Glu Glu Glu Asn Ala Lys
        210                 215                 220

cag atc ctc cag cgg ttg tgg ggc acc cag gat gta tcc caa gac atc      959
Gln Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile
    225                 230                 235

cag gag atg aaa gat gag agt gca agg atg tca caa gaa aag caa gtc     1007
Gln Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val
240                 245                 250                 255

acc gtg cta gag ctc ttt aga gtg tcc agc tac cga cag ccc atc atc     1055
Thr Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile
                260                 265                 270

att tcc att gtg ctc cag ctc tct cag cag ctc tct ggg atc aat gct     1103
Ile Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala
            275                 280                 285

gtg ttc tat tac tca aca gga atc ttc aag gat gca ggt gtt caa gag     1151
Val Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Glu
        290                 295                 300

ccc atc tat gcc acc atc ggc gcg ggt gtg gtt aat act atc ttc act     1199
Pro Ile Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Ile Phe Thr
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    305                 310                 315

gta gtt tct cta ttt ctg gtg gaa agg gca gga aga agg act ctg cat     1247
Val Val Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His
320                 325                 330                 335

atg ata ggc ctt gga ggg atg gct ttt tgt tcc acg ctc atg act gtt     1295
Met Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val
                340                 345                 350

tct ttg tta tta aag gat aac tat aat ggg atg agc ttt gtc tgt att     1343
Ser Leu Leu Leu Lys Asp Asn Tyr Asn Gly Met Ser Phe Val Cys Ile
            355                 360                 365

ggg gct atc ttg gtc ttt gta gcc ttc ttt gaa att gga cca ggc ccc     1391
Gly Ala Ile Leu Val Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro
        370                 375                 380

att ccc tgg ttt att gtg gcc gaa ctc ttc agc cag ggc ccc cgc cca     1439
Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro
    385                 390                 395

gct gcg atg gca gtg gcc ggc tgc tcc aac tgg acc tcc aac ttc cta     1487
Ala Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu
400                 405                 410                 415

gtc gga ttg ctc ttc ccc tcc gct gct cac tat tta gga gcc tac gtt     1535
Val Gly Leu Leu Phe Pro Ser Ala Ala His Tyr Leu Gly Ala Tyr Val
                420                 425                 430

ttt att atc ttc acc ggc ttc ctc att acc ttc ttg gct ttt acc ttc     1583
Phe Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe
            435                 440                 445

ttc aaa gtc cct gag acc cgt ggc agg act ttt gag gat atc aca cgg     1631
Phe Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg
        450                 455                 460

gcc ttt gaa ggg cag gca cac ggt gca gat aga tct gga aag gac ggc     1679
Ala Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly
    465                 470                 475

gtc atg gag atg aac agc atc gag cct gct aag gag acc acc acc aat     1727
Val Met Glu Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn
480                 485                 490                 495

gtc taa gtcgtgcctc cttccacctc cctcccggca tgggaaagcc acctctccct     1783
Val

caacaaggga gagacctcat caggatgaac ccaggacgct tctgaatgct gctacttaat   1843

tcctttctca tcccacgcac tccatgagca ccccaaggct gcggtttgtt ggatcttcaa   1903

tggcttttta aattttattt cctggacatc ctcttctgct taggagagac cgagtgaacc   1963

taccttcatt tcaggaggga ttggccgctt ggcacatgac aactttgcca gcttttcctc   2023

ccttgggttc tgatattgcc gcactagggg atataggaga ggaaaagtaa ggtgcagttc   2083

ccccaacctc agacttacca ggaagcagat acatatgagt gtggaagccg gagggtgttt   2143

atgtaagagc accttcctca cttccataca gctctacgtg gcaaattaac ttgagtttta   2203
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ctatgccgaa tgccctcagg ggacttgaat ctttccaata aaccaggttt agacagtatg 3163

agtcaatgtg cagtgtagcc cacacttgag aggatgaatg tatgtgcact gtcactttgc 3223

tctgggtgga agtacgttat tgttgactta ttttctctgt gtttgttcct acagcccctt 3283

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gccaaaccct tgtcagtgac agtattttgg ttcttagttc tcactgttcc ctctgctcct 3403

ggagcctttg aataaaaatg cacgtagctg aggccggatg cggtggctca cgcctgtaat 3463

cccagcactt tgggaggcct aggcgggcgg tcaggggttc gagaccagtc tggccaacat 3523

cgtgaaaccc tgtctctact aaaaatgcaa aaattagccg ggcgtggtgg cgggcgcctg 3583

taatcccagc tacttgggaa gctgaggcgg gagaatcatg tgaacccggg acgcaggggt 3643

tgcagtgagc ggagatcgca tcattgcact ctagcctggg ccacagggcg agactccgtc 3703

tcaaaaaaaa aaaaatgcac atagctatcg agtgtgcttt agcttgaaaa ggtgaccttg 3763

caacttcatg tcaactttct ggctcctcaa acagtaggtt ggcagtaagg cagggtccca 3823

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Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile Asn
            20                  25                  30

Ala Pro Glu Lys Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr Asp
        35                  40                  45

Lys Gly Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Ser Leu Trp Ser
    50                  55                  60

Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser
65                  70                  75                  80

Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Ile
                85                  90                  95

Val Asn Leu Leu Ala Val Thr Gly Gly Cys Phe Met Gly Leu Cys Lys
            100                 105                 110

Val Ala Lys Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile Gly
        115                 120                 125

Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly Glu
    130                 135                 140

Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln Leu
145                 150                 155                 160

Gly Ile Val Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu Phe
                165                 170                 175

Ile Leu Gly Ser Glu Glu Leu Trp Pro Leu Leu Leu Gly Phe Thr Ile
            180                 185                 190

Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Phe Cys Pro Glu Ser
        195                 200                 205

Pro Arg Phe Leu Leu Ile Asn Arg Lys Glu Glu Glu Asn Ala Lys Gln
    210                 215                 220

Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile Gln
225                 230                 235                 240
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Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val Thr
245 250 255

Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile Ile
260 265 270

Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val
275 280 285

Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Glu Pro
290 295 300

Ile Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr Ile Phe Thr Val
305 310 315 320

Val Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Met
325 330 335

Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val Ser
340 345 350

Leu Leu Leu Lys Asp Asn Tyr Asn Gly Met Ser Phe Val Cys Ile Gly
355 360 365

Ala Ile Leu Val Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile
370 375 380

Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala
385 390 395 400

Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu Val
405 410 415

Gly Leu Leu Phe Pro Ser Ala Ala His Tyr Leu Gly Ala Tyr Val Phe
420 425 430

Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe Phe
435 440 445

Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg Ala
450 455 460

Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly Val
465 470 475 480

Met Glu Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
485 490 495

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<212> DNA
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<221> CDS
<222> (1)..(1581)
<223>

<400> 40

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Leu Leu Ser Pro Phe Ser Ala Arg Val Thr Leu Ala Lys Arg Arg
1               5                   10                  15

gcg gca ctc tgc cct tcc aga gca agc atg gag caa cag gat cag agc       96
Ala Ala Leu Cys Pro Ser Arg Ala Ser Met Glu Gln Gln Asp Gln Ser
                20                  25                  30

atg aag gaa ggg agg ctg acg ctt gtg ctt gcc ctg gca acc ctg ata      144
Met Lys Glu Gly Arg Leu Thr Leu Val Leu Ala Leu Ala Thr Leu Ile
            35                  40                  45

gct gcc ttt ggg tca tcc ttc cag tat ggg tac aac gtg gct gct gtc      192
Ala Ala Phe Gly Ser Ser Phe Gln Tyr Gly Tyr Asn Val Ala Ala Val
        50                  55                  60

aac tcc cca gca ctg ctc atg caa caa ttt tac aat gag act tac tat      240
Asn Ser Pro Ala Leu Leu Met Gln Gln Phe Tyr Asn Glu Thr Tyr Tyr
    65                  70                  75

ggt agg acc ggt gaa ttc atg gaa gac ttc ccc ttg acg ttg ctg tgg      288
Gly Arg Thr Gly Glu Phe Met Glu Asp Phe Pro Leu Thr Leu Leu Trp
80                  85                  90                  95

tct gta acc gtg tcc atg ttt cca ttt gga ggg ttt atc gga tcc ctc      336
Ser Val Thr Val Ser Met Phe Pro Phe Gly Gly Phe Ile Gly Ser Leu
                100                 105                 110

ctg gtc ggc ccc ttg gtg aat aaa ttt ggc aga aaa ggg gcc ttg ctg      384
Leu Val Gly Pro Leu Val Asn Lys Phe Gly Arg Lys Gly Ala Leu Leu
            115                 120                 125

ttc aac aac ata ttt tct atc gtg cct gcg atc tta atg gga tgc agc      432
Phe Asn Asn Ile Phe Ser Ile Val Pro Ala Ile Leu Met Gly Cys Ser
        130                 135                 140

aga gtc gcc aca tca ttt gag ctt atc att att tcc aga ctt ttg gtg      480
Arg Val Ala Thr Ser Phe Glu Leu Ile Ile Ile Ser Arg Leu Leu Val
    145                 150                 155

gga ata tgt gca ggt gta tct tcc aac gtg gtc ccc atg tac tta ggg      528
Gly Ile Cys Ala Gly Val Ser Ser Asn Val Val Pro Met Tyr Leu Gly
160                 165                 170                 175

gag ctg gcc cct aaa aac ctg cgg ggg gct ctc ggg gtg gtg ccc cag      576
Glu Leu Ala Pro Lys Asn Leu Arg Gly Ala Leu Gly Val Val Pro Gln
                180                 185                 190

ctc ttc atc act gtt ggc atc ctt gtg gcc cag atc ttt ggt ctt cgg      624
Leu Phe Ile Thr Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Arg
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                195                 200                 205

aat ctc ctt gca aac gta gat ggc tgg ccg atc ctg ctg ggg ctg acc      672
Asn Leu Leu Ala Asn Val Asp Gly Trp Pro Ile Leu Leu Gly Leu Thr
        210                 215                 220

ggg gtc ccc gcg gcg ctg cag ctc ctt ctg ctg ccc ttc ttc ccc gag      720
Gly Val Pro Ala Ala Leu Gln Leu Leu Leu Leu Pro Phe Phe Pro Glu
    225                 230                 235

agc ccc agg tac ctg ctg att cag aag aaa gac gaa gcg gcc gcc aag      768
Ser Pro Arg Tyr Leu Leu Ile Gln Lys Lys Asp Glu Ala Ala Ala Lys
240                 245                 250                 255

aaa gcc cta cag acg ctg cgc ggc tgg gac tct gtg gac agg gag gtg      816
Lys Ala Leu Gln Thr Leu Arg Gly Trp Asp Ser Val Asp Arg Glu Val
                260                 265                 270

gcc gag atc cgg cag gag gat gag gca gag aag gcc gcg ggc ttc atc      864
Ala Glu Ile Arg Gln Glu Asp Glu Ala Glu Lys Ala Ala Gly Phe Ile
            275                 280                 285

tcc gtg ctg aag ctg ttc cgg atg cgc tcg ctg cgc tgg cag ctg ctg      912
Ser Val Leu Lys Leu Phe Arg Met Arg Ser Leu Arg Trp Gln Leu Leu
        290                 295                 300

tcc atc atc gtc ctc atg ggc ggc cag cag ctg tcg ggc gtc aac gct      960
Ser Ile Ile Val Leu Met Gly Gly Gln Gln Leu Ser Gly Val Asn Ala
    305                 310                 315

atc tac tac tac gcg gac cag atc tac ctg agc gcc ggc gtg ccg gag     1008
Ile Tyr Tyr Tyr Ala Asp Gln Ile Tyr Leu Ser Ala Gly Val Pro Glu
320                 325                 330                 335

gag cac gtg cag tac gtg acg gcc ggc acc ggg gcc gtg aac gtg gtc     1056
Glu His Val Gln Tyr Val Thr Ala Gly Thr Gly Ala Val Asn Val Val
                340                 345                 350

atg acc ttc tgc gcc gtg ttc gtg gtg gag ctc ctg ggt cgg agg ctg     1104
Met Thr Phe Cys Ala Val Phe Val Val Glu Leu Leu Gly Arg Arg Leu
            355                 360                 365

ctg ctg ctg ctg ggc ttc tcc atc tgc ctc ata gcc tgc tgc gtg ctc     1152
Leu Leu Leu Leu Gly Phe Ser Ile Cys Leu Ile Ala Cys Cys Val Leu
        370                 375                 380

act gca gct ctg gca ctg cag gac aca gtg tcc tgg atg cca tac atc     1200
Thr Ala Ala Leu Ala Leu Gln Asp Thr Val Ser Trp Met Pro Tyr Ile
    385                 390                 395

agc atc gtc tgt gtc atc tcc tac gtc ata gga cat gcc ctc ggg ccc     1248
Ser Ile Val Cys Val Ile Ser Tyr Val Ile Gly His Ala Leu Gly Pro
400                 405                 410                 415

agt ccc ata ccc gcg ctg ctc atc act gag atc ttc ctg cag tcc tct     1296
Ser Pro Ile Pro Ala Leu Leu Ile Thr Glu Ile Phe Leu Gln Ser Ser
                420                 425                 430

cgg cca tct gcc ttc atg gtg ggg ggc agt gtg cac tgg ctc tcc aac     1344
Arg Pro Ser Ala Phe Met Val Gly Gly Ser Val His Trp Leu Ser Asn
            435                 440                 445

ttc acc gtg ggc ttg atc ttc ccg ttc atc cag gag ggc ctc ggc ccg     1392
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Phe Thr Val Gly Leu Ile Phe Pro Phe Ile Gln Glu Gly Leu Gly Pro
        450                 455                 460

tac agc ttc att gtc ttc gcc gtg atc tgc ctc ctc acc acc atc tac     1440
Tyr Ser Phe Ile Val Phe Ala Val Ile Cys Leu Leu Thr Thr Ile Tyr
    465                 470                 475

atc ttc ttg att gtc ccg gag acc aag gcc aag acg ttc ata gag atc     1488
Ile Phe Leu Ile Val Pro Glu Thr Lys Ala Lys Thr Phe Ile Glu Ile
480                 485                 490                 495

aac cag att ttc acc aag atg aat aag gtg tct gaa gtg tac ccg gaa     1536
Asn Gln Ile Phe Thr Lys Met Asn Lys Val Ser Glu Val Tyr Pro Glu
                500                 505                 510

aag gag gaa ctg aaa gag ctt cca cct gtc act tcg gaa cag tga         1581
Lys Glu Glu Leu Lys Glu Leu Pro Pro Val Thr Ser Glu Gln
            515                 520                 525

ctctggagag gaagccagtg gagctggtct gccaggggct tcccactttg gcttattttt   1641

ctgacttcta gctgtctgtg aatatccaga aataaaacaa ctctgatgtg gaatgcagtc   1701

ctcatctcca gcctccccac cccagtggga actgtgcaaa gggctgcctt gctgttcttg   1761

aagctgggct gtctctctcc atgttggcct gtcaccagac ccgagtcaat taaacagctg   1821

gtcctccact ttgctggttc agccttcgtg tggctcctgg taacgtggct ccaccttgat   1881

gggtcaacct ttgtgtggct cctggtaaca taacaacaac agttactata gtggtgagat   1941

ggaaggaatc aaattttgcc agagaaacta actcggtggc cccaacaggt cttccggggc   2001

catgggcatt tgtttagagc caaattcatc ctcttaccag atccttttcc agaaatacct   2061

gtctaggaag gtgtgatgtc agaaacaatg acatccagaa agctgaggaa caggttcctg   2121

tggagacact gagtcagaat tcttcatcca aattattttg ttagtggaaa atggaattgc   2181

ttctgtgtag tcaataaaat gaacctgatc acttttc                            2218
```

<210> 41
<211> 15
<212> PRT
<213> GLUT5

<400> 41

```
Leu Leu Ser Pro Phe Ser Ala Arg Val Thr Leu Ala Lys Arg Arg
1               5                   10                  15
```

<210> 42
<211> 510
<212> PRT
<213> GLUT5

<400> 42

```
Ala Ala Leu Cys Pro Ser Arg Ala Ser Met Glu Gln Gln Asp Gln Ser
1               5                   10                  15
```

```
Met Lys Glu Gly Arg Leu Thr Leu Val Leu Ala Leu Ala Thr Leu Ile
            20                  25                  30

Ala Ala Phe Gly Ser Ser Phe Gln Tyr Gly Tyr Asn Val Ala Ala Val
        35                  40                  45

Asn Ser Pro Ala Leu Leu Met Gln Gln Phe Tyr Asn Glu Thr Tyr Tyr
    50                  55                  60

Gly Arg Thr Gly Glu Phe Met Glu Asp Phe Pro Leu Thr Leu Leu Trp
65                  70                  75                  80

Ser Val Thr Val Ser Met Phe Pro Phe Gly Gly Phe Ile Gly Ser Leu
                85                  90                  95

Leu Val Gly Pro Leu Val Asn Lys Phe Gly Arg Lys Gly Ala Leu Leu
            100                 105                 110

Phe Asn Asn Ile Phe Ser Ile Val Pro Ala Ile Leu Met Gly Cys Ser
        115                 120                 125

Arg Val Ala Thr Ser Phe Glu Leu Ile Ile Ile Ser Arg Leu Leu Val
    130                 135                 140

Gly Ile Cys Ala Gly Val Ser Ser Asn Val Val Pro Met Tyr Leu Gly
145                 150                 155                 160

Glu Leu Ala Pro Lys Asn Leu Arg Gly Ala Leu Gly Val Val Pro Gln
                165                 170                 175

Leu Phe Ile Thr Val Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Arg
            180                 185                 190

Asn Leu Leu Ala Asn Val Asp Gly Trp Pro Ile Leu Leu Gly Leu Thr
        195                 200                 205

Gly Val Pro Ala Ala Leu Gln Leu Leu Leu Leu Pro Phe Phe Pro Glu
    210                 215                 220

Ser Pro Arg Tyr Leu Leu Ile Gln Lys Lys Asp Glu Ala Ala Ala Lys
225                 230                 235                 240

Lys Ala Leu Gln Thr Leu Arg Gly Trp Asp Ser Val Asp Arg Glu Val
                245                 250                 255

Ala Glu Ile Arg Gln Glu Asp Glu Ala Glu Lys Ala Ala Gly Phe Ile
            260                 265                 270
```

```
Ser Val Leu Lys Leu Phe Arg Met Arg Ser Leu Arg Trp Gln Leu Leu
        275                 280                 285

Ser Ile Ile Val Leu Met Gly Gly Gln Gln Leu Ser Gly Val Asn Ala
    290                 295                 300

Ile Tyr Tyr Tyr Ala Asp Gln Ile Tyr Leu Ser Ala Gly Val Pro Glu
305                 310                 315                 320

Glu His Val Gln Tyr Val Thr Ala Gly Thr Gly Ala Val Asn Val Val
                325                 330                 335

Met Thr Phe Cys Ala Val Phe Val Val Glu Leu Leu Gly Arg Arg Leu
            340                 345                 350

Leu Leu Leu Leu Gly Phe Ser Ile Cys Leu Ile Ala Cys Cys Val Leu
        355                 360                 365

Thr Ala Ala Leu Ala Leu Gln Asp Thr Val Ser Trp Met Pro Tyr Ile
    370                 375                 380

Ser Ile Val Cys Val Ile Ser Tyr Val Ile Gly His Ala Leu Gly Pro
385                 390                 395                 400

Ser Pro Ile Pro Ala Leu Leu Ile Thr Glu Ile Phe Leu Gln Ser Ser
                405                 410                 415

Arg Pro Ser Ala Phe Met Val Gly Gly Ser Val His Trp Leu Ser Asn
            420                 425                 430

Phe Thr Val Gly Leu Ile Phe Pro Phe Ile Gln Glu Gly Leu Gly Pro
        435                 440                 445

Tyr Ser Phe Ile Val Phe Ala Val Ile Cys Leu Leu Thr Thr Ile Tyr
    450                 455                 460

Ile Phe Leu Ile Val Pro Glu Thr Lys Ala Lys Thr Phe Ile Glu Ile
465                 470                 475                 480

Asn Gln Ile Phe Thr Lys Met Asn Lys Val Ser Glu Val Tyr Pro Glu
                485                 490                 495

Lys Glu Glu Leu Lys Glu Leu Pro Pro Val Thr Ser Glu Gln
            500                 505                 510
```

<210> 43

<211> 1580
<212> DNA
<213> GLUT6

<220>
<221> CDS
<222> (47)..(1570)
<223>

<400> 43

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tgctccagtc tgagcgccct ccgctcgccc cgagagagac ccggcc atg cag gag        55
                                                    Met Gln Glu
                                                    1

ccg ctg ctg gga gcc gag ggc ccg gac tac gac acc ttc ccc gag aag      103
Pro Leu Leu Gly Ala Glu Gly Pro Asp Tyr Asp Thr Phe Pro Glu Lys
    5                  10                  15

ccg ccc ccg tcg cca ggg gac agg gcg cgg gtc ggg acc ctg cag aac      151
Pro Pro Pro Ser Pro Gly Asp Arg Ala Arg Val Gly Thr Leu Gln Asn
20                 25                  30                  35

aaa agg gtg ttc ctg gcc acc ttc gcc gca gtg ctc ggc aat ttc agc      199
Lys Arg Val Phe Leu Ala Thr Phe Ala Ala Val Leu Gly Asn Phe Ser
               40                  45                  50

ttt ggg tat gcc ctg gtc tac aca tcc cct gtc atc cca gcc ctg gag      247
Phe Gly Tyr Ala Leu Val Tyr Thr Ser Pro Val Ile Pro Ala Leu Glu
           55                  60                  65

cgc tcc ttg gat cct gac ctg cat ctg acc aaa tcc cag gca tcc tgg      295
Arg Ser Leu Asp Pro Asp Leu His Leu Thr Lys Ser Gln Ala Ser Trp
       70                  75                  80

ttt ggg tcc gtg ttc acc ctg gga gca gcg gcc gga ggc ctg agt gcc      343
Phe Gly Ser Val Phe Thr Leu Gly Ala Ala Ala Gly Gly Leu Ser Ala
    85                  90                  95

atg atc ctc aac gac ctc ctg ggc cgg aag ctg agc atc atg ttc tca      391
Met Ile Leu Asn Asp Leu Leu Gly Arg Lys Leu Ser Ile Met Phe Ser
100                 105                 110                 115

gct gtg ccg tcg gcg gcc ggc tat gcg ctc atg gcg ggt gcg cac ggc      439
Ala Val Pro Ser Ala Ala Gly Tyr Ala Leu Met Ala Gly Ala His Gly
               120                 125                 130

ctc tgg atg ctg ctg ctc gga agg acg ctg acg ggc ttc gcc ggg ggg      487
Leu Trp Met Leu Leu Leu Gly Arg Thr Leu Thr Gly Phe Ala Gly Gly
           135                 140                 145

ctc aca gct gcc tgc atc ccg gtg tac gtg tct gag att gct ccc cca      535
Leu Thr Ala Ala Cys Ile Pro Val Tyr Val Ser Glu Ile Ala Pro Pro
       150                 155                 160

ggc gtt cgt ggg gct ctg ggg gcc aca ccc cag ctc atg gca gtg ttc      583
Gly Val Arg Gly Ala Leu Gly Ala Thr Pro Gln Leu Met Ala Val Phe
    165                 170                 175

gga tcc ctg tcc ctc tac gcc ctt ggc ctc ctg ctg ccg tgg cgc tgg      631
Gly Ser Leu Ser Leu Tyr Ala Leu Gly Leu Leu Leu Pro Trp Arg Trp
180                 185                 190                 195

ctg gct gtg gcc ggg gag gcg cct gtg ctc atc atg atc ctg ctg ctc      679
```

```
Leu Ala Val Ala Gly Glu Ala Pro Val Leu Ile Met Ile Leu Leu Leu
                200                 205                 210

agc ttc atg ccc aac tcg ccg cgc ttc ctg ctc tct cgg ggc agg gac      727
Ser Phe Met Pro Asn Ser Pro Arg Phe Leu Leu Ser Arg Gly Arg Asp
            215                 220                 225

gaa gag gcc ctg cgg gcg ctg gcc tgg ctg cgt ggg acg gac gtc gat      775
Glu Glu Ala Leu Arg Ala Leu Ala Trp Leu Arg Gly Thr Asp Val Asp
        230                 235                 240

gtc cac tgg gag ttc gag cag atc cag gac aac gtc cgg aga cag agc      823
Val His Trp Glu Phe Glu Gln Ile Gln Asp Asn Val Arg Arg Gln Ser
    245                 250                 255

agc cga gta tcg tgg gct gag gca cgg gcc cca cac gtg tgc cgg ccc      871
Ser Arg Val Ser Trp Ala Glu Ala Arg Ala Pro His Val Cys Arg Pro
260                 265                 270                 275

atc acc gtg gcc ttg ctg atg cgc ctc ctg cag cag ctg acg ggc atc      919
Ile Thr Val Ala Leu Leu Met Arg Leu Leu Gln Gln Leu Thr Gly Ile
                280                 285                 290

acg ccc atc ctg gtc tac ctg cag tcc atc ttc gac agc acc gct gtc      967
Thr Pro Ile Leu Val Tyr Leu Gln Ser Ile Phe Asp Ser Thr Ala Val
            295                 300                 305

ctg ctg ccc ccc aag gac gac gca gcc atc gtt ggg gcc gtg cgg ctc     1015
Leu Leu Pro Pro Lys Asp Asp Ala Ala Ile Val Gly Ala Val Arg Leu
        310                 315                 320

ctg tcc gtg ctg atc gcc gcc ctc acc atg gac ctc gca ggc cgc aag     1063
Leu Ser Val Leu Ile Ala Ala Leu Thr Met Asp Leu Ala Gly Arg Lys
    325                 330                 335

gtg ctg ctc ttc gtc tca gcg gcc atc atg ttt gct gcc aac ctg act     1111
Val Leu Leu Phe Val Ser Ala Ala Ile Met Phe Ala Ala Asn Leu Thr
340                 345                 350                 355

ctg ggg ctg tac atc cac ttt ggc ccc agg cct ctg agc ccc aac agc     1159
Leu Gly Leu Tyr Ile His Phe Gly Pro Arg Pro Leu Ser Pro Asn Ser
                360                 365                 370

act gcg ggc ctg gaa agc gag tcc tgg ggg gac ttg gcg cag ccc ctg     1207
Thr Ala Gly Leu Glu Ser Glu Ser Trp Gly Asp Leu Ala Gln Pro Leu
            375                 380                 385

gca gca ccc gct ggc tac ctc acc ctg gtg ccc ctg ctg gcc acc atg     1255
Ala Ala Pro Ala Gly Tyr Leu Thr Leu Val Pro Leu Leu Ala Thr Met
        390                 395                 400

ctc ttc atc atg ggc tac gcc gtg ggc tgg ggt ccc atc acc tgg ctg     1303
Leu Phe Ile Met Gly Tyr Ala Val Gly Trp Gly Pro Ile Thr Trp Leu
    405                 410                 415

ctc atg tct gag gtc ctg ccc ctg cgt gcc cgt ggc gtg gcc tca ggg     1351
Leu Met Ser Glu Val Leu Pro Leu Arg Ala Arg Gly Val Ala Ser Gly
420                 425                 430                 435

ctc tgc gtg ctg gcc agc tgg ctc acc gcc ttc gtc ctc acc aag tcc     1399
Leu Cys Val Leu Ala Ser Trp Leu Thr Ala Phe Val Leu Thr Lys Ser
                440                 445                 450
```

```
ttc ctg cca gtg gtg agc acc ttc ggc ctc cag gtg cct ttc ttc ttc     1447
Phe Leu Pro Val Val Ser Thr Phe Gly Leu Gln Val Pro Phe Phe Phe
            455                 460                 465

ttc gcg gcc atc tgc ttg gtg agc ctg gtg ttc aca ggc tgc tgt gtg     1495
Phe Ala Ala Ile Cys Leu Val Ser Leu Val Phe Thr Gly Cys Cys Val
        470                 475                 480

ccc gag acc aag gga cgg tcc ctg gag cag atc gag tcc ttc ttc cgc     1543
Pro Glu Thr Lys Gly Arg Ser Leu Glu Gln Ile Glu Ser Phe Phe Arg
    485                 490                 495

atg ggg aga agg tcc ttc ttg cgc tag gtcaaggtcc                      1580
Met Gly Arg Arg Ser Phe Leu Arg
500                 505
```

<210> 44
<211> 507
<212> PRT
<213> GLUT6

<400> 44

```
Met Gln Glu Pro Leu Leu Gly Ala Glu Gly Pro Asp Tyr Asp Thr Phe
1               5                   10                  15

Pro Glu Lys Pro Pro Pro Ser Pro Gly Asp Arg Ala Arg Val Gly Thr
            20                  25                  30

Leu Gln Asn Lys Arg Val Phe Leu Ala Thr Phe Ala Ala Val Leu Gly
        35                  40                  45

Asn Phe Ser Phe Gly Tyr Ala Leu Val Tyr Thr Ser Pro Val Ile Pro
    50                  55                  60

Ala Leu Glu Arg Ser Leu Asp Pro Asp Leu His Leu Thr Lys Ser Gln
65                  70                  75                  80

Ala Ser Trp Phe Gly Ser Val Phe Thr Leu Gly Ala Ala Ala Gly Gly
                85                  90                  95

Leu Ser Ala Met Ile Leu Asn Asp Leu Leu Gly Arg Lys Leu Ser Ile
            100                 105                 110

Met Phe Ser Ala Val Pro Ser Ala Ala Gly Tyr Ala Leu Met Ala Gly
        115                 120                 125

Ala His Gly Leu Trp Met Leu Leu Leu Gly Arg Thr Leu Thr Gly Phe
    130                 135                 140

Ala Gly Gly Leu Thr Ala Ala Cys Ile Pro Val Tyr Val Ser Glu Ile
145                 150                 155                 160
```

```
Ala Pro Pro Gly Val Arg Gly Ala Leu Gly Ala Thr Pro Gln Leu Met
                165                 170                 175

Ala Val Phe Gly Ser Leu Ser Leu Tyr Ala Leu Gly Leu Leu Leu Pro
            180                 185                 190

Trp Arg Trp Leu Ala Val Ala Gly Glu Ala Pro Val Leu Ile Met Ile
        195                 200                 205

Leu Leu Leu Ser Phe Met Pro Asn Ser Pro Arg Phe Leu Leu Ser Arg
    210                 215                 220

Gly Arg Asp Glu Glu Ala Leu Arg Ala Leu Ala Trp Leu Arg Gly Thr
225                 230                 235                 240

Asp Val Asp Val His Trp Glu Phe Glu Gln Ile Gln Asp Asn Val Arg
                245                 250                 255

Arg Gln Ser Ser Arg Val Ser Trp Ala Glu Ala Arg Ala Pro His Val
            260                 265                 270

Cys Arg Pro Ile Thr Val Ala Leu Leu Met Arg Leu Leu Gln Gln Leu
        275                 280                 285

Thr Gly Ile Thr Pro Ile Leu Val Tyr Leu Gln Ser Ile Phe Asp Ser
    290                 295                 300

Thr Ala Val Leu Leu Pro Pro Lys Asp Asp Ala Ala Ile Val Gly Ala
305                 310                 315                 320

Val Arg Leu Leu Ser Val Leu Ile Ala Ala Leu Thr Met Asp Leu Ala
                325                 330                 335

Gly Arg Lys Val Leu Leu Phe Val Ser Ala Ala Ile Met Phe Ala Ala
            340                 345                 350

Asn Leu Thr Leu Gly Leu Tyr Ile His Phe Gly Pro Arg Pro Leu Ser
        355                 360                 365

Pro Asn Ser Thr Ala Gly Leu Glu Ser Glu Ser Trp Gly Asp Leu Ala
    370                 375                 380

Gln Pro Leu Ala Ala Pro Ala Gly Tyr Leu Thr Leu Val Pro Leu Leu
385                 390                 395                 400

Ala Thr Met Leu Phe Ile Met Gly Tyr Ala Val Gly Trp Gly Pro Ile
                405                 410                 415
```

```
Thr Trp Leu Leu Met Ser Glu Val Leu Pro Leu Arg Ala Arg Gly Val
            420                 425                 430

Ala Ser Gly Leu Cys Val Leu Ala Ser Trp Leu Thr Ala Phe Val Leu
        435                 440                 445

Thr Lys Ser Phe Leu Pro Val Val Ser Thr Phe Gly Leu Gln Val Pro
    450                 455                 460

Phe Phe Phe Phe Ala Ala Ile Cys Leu Val Ser Leu Val Phe Thr Gly
465                 470                 475                 480

Cys Cys Val Pro Glu Thr Lys Gly Arg Ser Leu Glu Gln Ile Glu Ser
                485                 490                 495

Phe Phe Arg Met Gly Arg Arg Ser Phe Leu Arg
            500                 505
```

<210> 45
<211> 1575
<212> DNA
<213> GLUT7

<220>
<221> CDS
<222> (1)..(1575)
<223>

<400> 45

```
atg gag aac aaa gag gcg gga acc cct cca ccc att cca tcc agg gag       48
Met Glu Asn Lys Glu Ala Gly Thr Pro Pro Pro Ile Pro Ser Arg Glu
1               5                   10                  15

ggg cgg ctc cag ccg acg ctg ttg ctg gcg aca ctg agc gcg gcc ttt       96
Gly Arg Leu Gln Pro Thr Leu Leu Leu Ala Thr Leu Ser Ala Ala Phe
            20                  25                  30

ggc tca gcc ttc cag tac ggc tac aac ctc tct gtg gtc aac acg ccg      144
Gly Ser Ala Phe Gln Tyr Gly Tyr Asn Leu Ser Val Val Asn Thr Pro
        35                  40                  45

cac aag gtg ggc aca agc tgt gga tgg ggc aat gtt ttc cag gtc ttc      192
His Lys Val Gly Thr Ser Cys Gly Trp Gly Asn Val Phe Gln Val Phe
    50                  55                  60

aag tca ttt tac aac gaa acc tac ttt gag cga cac gca aca ttc atg      240
Lys Ser Phe Tyr Asn Glu Thr Tyr Phe Glu Arg His Ala Thr Phe Met
65                  70                  75                  80

gac ggg aag ctc atg ctg ctt cta tgg tct tgc acc gtc tcc atg ttt      288
Asp Gly Lys Leu Met Leu Leu Leu Trp Ser Cys Thr Val Ser Met Phe
                85                  90                  95

cct ctg ggc ggc ctg ttg ggg tca ttg ctc gtg ggc ctg ctg gtt gat      336
Pro Leu Gly Gly Leu Leu Gly Ser Leu Leu Val Gly Leu Leu Val Asp
```

```
                100                 105                 110

agc tgc ggc aga aag ggg acc ctg ctg atc aac aac atc ttt gcc atc      384
Ser Cys Gly Arg Lys Gly Thr Leu Leu Ile Asn Asn Ile Phe Ala Ile
        115                 120                 125

atc ccc gcc atc ctg atg gga gtc agc aaa gtg gcc aag gct ttt gag      432
Ile Pro Ala Ile Leu Met Gly Val Ser Lys Val Ala Lys Ala Phe Glu
    130                 135                 140

ctg atc gtc ttt tcc cga gtg gtg ctg gga gtc tgt gca ggc atc tcc      480
Leu Ile Val Phe Ser Arg Val Val Leu Gly Val Cys Ala Gly Ile Ser
145                 150                 155                 160

tac agc gcc ctt ccc atg tac ctg gga gaa ctg gcc ccc aag aac ctg      528
Tyr Ser Ala Leu Pro Met Tyr Leu Gly Glu Leu Ala Pro Lys Asn Leu
                165                 170                 175

aga ggc atg gtg gga aca atg acc gag gtt ttc gtc atc gtt gga gtc      576
Arg Gly Met Val Gly Thr Met Thr Glu Val Phe Val Ile Val Gly Val
            180                 185                 190

ttc cta gca cag atc ttc agc ctc cag gcc atc ttg ggc aac ccg gca      624
Phe Leu Ala Gln Ile Phe Ser Leu Gln Ala Ile Leu Gly Asn Pro Ala
        195                 200                 205

ggc tgg ccg gtg ctt ctg gcg ctc aca ggg gtg ccc gcc ctg ctg cag      672
Gly Trp Pro Val Leu Leu Ala Leu Thr Gly Val Pro Ala Leu Leu Gln
    210                 215                 220

ctg ctg acc ctg ccc ttc ttc ccc gaa agc ccc cgc tac tcc ctg att      720
Leu Leu Thr Leu Pro Phe Phe Pro Glu Ser Pro Arg Tyr Ser Leu Ile
225                 230                 235                 240

cag aaa gga gat gaa gcc aca gcg cga caa gct ctg agg agg ctg aga      768
Gln Lys Gly Asp Glu Ala Thr Ala Arg Gln Ala Leu Arg Arg Leu Arg
                245                 250                 255

ggc cac acg gac atg gag gcc gag ctg gag gac atg cgt gcg gag gcc      816
Gly His Thr Asp Met Glu Ala Glu Leu Glu Asp Met Arg Ala Glu Ala
            260                 265                 270

cgg gcc gag cgc gcc gag ggc cac ctg tct gtg ctg cac ctc tgt gcc      864
Arg Ala Glu Arg Ala Glu Gly His Leu Ser Val Leu His Leu Cys Ala
        275                 280                 285

ctg cgg tcc ctg cgc tgg cag ctc ctc tcc atc atc gtg ctc atg gcc      912
Leu Arg Ser Leu Arg Trp Gln Leu Leu Ser Ile Ile Val Leu Met Ala
    290                 295                 300

ggc cag cag ctg tcg ggc atc aat gcg atc aac tac tat gcg gac acc      960
Gly Gln Gln Leu Ser Gly Ile Asn Ala Ile Asn Tyr Tyr Ala Asp Thr
305                 310                 315                 320

atc tac aca tct gcg ggc gtg gag gcc gct cac tcc caa tat gta acg     1008
Ile Tyr Thr Ser Ala Gly Val Glu Ala Ala His Ser Gln Tyr Val Thr
                325                 330                 335

gtg ggc tct ggc gtc gtc aac ata gtg atg acc atc acc tcg gct gtc     1056
Val Gly Ser Gly Val Val Asn Ile Val Met Thr Ile Thr Ser Ala Val
            340                 345                 350

ctt gtg gag cgg ctg gga cgg cgg cac ctc ctg ctg gcc ggc tac ggc     1104
```

```
Leu Val Glu Arg Leu Gly Arg Arg His Leu Leu Leu Ala Gly Tyr Gly
        355                 360                 365

atc tgc ggc tct gcc tgc ctg gtg ctg acg gtg gtg ctc cta ttc cag     1152
Ile Cys Gly Ser Ala Cys Leu Val Leu Thr Val Val Leu Leu Phe Gln
    370                 375                 380

aac agg gtc ccc gag ctg tcc tac ctc ggc atc atc tgt gtc ttt gcc     1200
Asn Arg Val Pro Glu Leu Ser Tyr Leu Gly Ile Ile Cys Val Phe Ala
385                 390                 395                 400

tac atc gcg gga cat tcc att ggg ccc agt cct gtc ccc tcg gtg gtg     1248
Tyr Ile Ala Gly His Ser Ile Gly Pro Ser Pro Val Pro Ser Val Val
                405                 410                 415

agg acc gag atc ttc ctg cag tcc tcc cgg cgg gca gct ttc atg gtg     1296
Arg Thr Glu Ile Phe Leu Gln Ser Ser Arg Arg Ala Ala Phe Met Val
            420                 425                 430

gac ggg gca gtg cac tgg ctc acc aac ttc atc ata ggc ttc ctg ttc     1344
Asp Gly Ala Val His Trp Leu Thr Asn Phe Ile Ile Gly Phe Leu Phe
        435                 440                 445

cca tcc atc cag gag gcc atc ggt gcc tac agt ttc atc atc ttt gcc     1392
Pro Ser Ile Gln Glu Ala Ile Gly Ala Tyr Ser Phe Ile Ile Phe Ala
    450                 455                 460

gga atc tgc ctc ctc act gcg att tac atc tac gtg gtt att ccg gag     1440
Gly Ile Cys Leu Leu Thr Ala Ile Tyr Ile Tyr Val Val Ile Pro Glu
465                 470                 475                 480

acc aag ggc aaa aca ttt gtg gag ata aac cgc att ttt gcc aag aga     1488
Thr Lys Gly Lys Thr Phe Val Glu Ile Asn Arg Ile Phe Ala Lys Arg
                485                 490                 495

aac agg gtg aag ctt cca gag gag aaa gaa gaa acc att gat gct ggg     1536
Asn Arg Val Lys Leu Pro Glu Glu Lys Glu Glu Thr Ile Asp Ala Gly
            500                 505                 510

cct ccc aca gcc tct cct gcc aag gaa act tcc ttt tag                 1575
Pro Pro Thr Ala Ser Pro Ala Lys Glu Thr Ser Phe
        515                 520


<210>  46
<211>  524
<212>  PRT
<213>  GLUT7

<400>  46

Met Glu Asn Lys Glu Ala Gly Thr Pro Pro Pro Ile Pro Ser Arg Glu
1               5                   10                  15


Gly Arg Leu Gln Pro Thr Leu Leu Leu Ala Thr Leu Ser Ala Ala Phe
            20                  25                  30


Gly Ser Ala Phe Gln Tyr Gly Tyr Asn Leu Ser Val Val Asn Thr Pro
        35                  40                  45
```

His Lys Val Gly Thr Ser Cys Gly Trp Gly Asn Val Phe Gln Val Phe
50 55 60

Lys Ser Phe Tyr Asn Glu Thr Tyr Phe Glu Arg His Ala Thr Phe Met
65 70 75 80

Asp Gly Lys Leu Met Leu Leu Leu Trp Ser Cys Thr Val Ser Met Phe
85 90 95

Pro Leu Gly Gly Leu Leu Gly Ser Leu Leu Val Gly Leu Leu Val Asp
100 105 110

Ser Cys Gly Arg Lys Gly Thr Leu Leu Ile Asn Asn Ile Phe Ala Ile
115 120 125

Ile Pro Ala Ile Leu Met Gly Val Ser Lys Val Ala Lys Ala Phe Glu
130 135 140

Leu Ile Val Phe Ser Arg Val Val Leu Gly Val Cys Ala Gly Ile Ser
145 150 155 160

Tyr Ser Ala Leu Pro Met Tyr Leu Gly Glu Leu Ala Pro Lys Asn Leu
165 170 175

Arg Gly Met Val Gly Thr Met Thr Glu Val Phe Val Ile Val Gly Val
180 185 190

Phe Leu Ala Gln Ile Phe Ser Leu Gln Ala Ile Leu Gly Asn Pro Ala
195 200 205

Gly Trp Pro Val Leu Leu Ala Leu Thr Gly Val Pro Ala Leu Leu Gln
210 215 220

Leu Leu Thr Leu Pro Phe Phe Pro Glu Ser Pro Arg Tyr Ser Leu Ile
225 230 235 240

Gln Lys Gly Asp Glu Ala Thr Ala Arg Gln Ala Leu Arg Arg Leu Arg
245 250 255

Gly His Thr Asp Met Glu Ala Glu Leu Glu Asp Met Arg Ala Glu Ala
260 265 270

Arg Ala Glu Arg Ala Glu Gly His Leu Ser Val Leu His Leu Cys Ala
275 280 285

Leu Arg Ser Leu Arg Trp Gln Leu Leu Ser Ile Ile Val Leu Met Ala
290 295 300

```
Gly Gln Gln Leu Ser Gly Ile Asn Ala Ile Asn Tyr Tyr Ala Asp Thr
305                 310                 315                 320

Ile Tyr Thr Ser Ala Gly Val Glu Ala Ala His Ser Gln Tyr Val Thr
                325                 330                 335

Val Gly Ser Gly Val Val Asn Ile Val Met Thr Ile Thr Ser Ala Val
            340                 345                 350

Leu Val Glu Arg Leu Gly Arg Arg His Leu Leu Leu Ala Gly Tyr Gly
        355                 360                 365

Ile Cys Gly Ser Ala Cys Leu Val Leu Thr Val Val Leu Leu Phe Gln
    370                 375                 380

Asn Arg Val Pro Glu Leu Ser Tyr Leu Gly Ile Ile Cys Val Phe Ala
385                 390                 395                 400

Tyr Ile Ala Gly His Ser Ile Gly Pro Ser Pro Val Pro Ser Val Val
                405                 410                 415

Arg Thr Glu Ile Phe Leu Gln Ser Ser Arg Arg Ala Ala Phe Met Val
            420                 425                 430

Asp Gly Ala Val His Trp Leu Thr Asn Phe Ile Ile Gly Phe Leu Phe
        435                 440                 445

Pro Ser Ile Gln Glu Ala Ile Gly Ala Tyr Ser Phe Ile Ile Phe Ala
    450                 455                 460

Gly Ile Cys Leu Leu Thr Ala Ile Tyr Ile Tyr Val Val Ile Pro Glu
465                 470                 475                 480

Thr Lys Gly Lys Thr Phe Val Glu Ile Asn Arg Ile Phe Ala Lys Arg
                485                 490                 495

Asn Arg Val Lys Leu Pro Glu Glu Lys Glu Glu Thr Ile Asp Ala Gly
            500                 505                 510

Pro Pro Thr Ala Ser Pro Ala Lys Glu Thr Ser Phe
        515                 520
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<210> 47
<211> 1508
<212> DNA
<213> GLUT8

<220>

<221> CDS
<222> (27)..(1460)
<223>

<400> 47

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agctggccga tgcgttggcc gccgac atg acg ccc gag gac cca gag gaa acc      53
                             Met Thr Pro Glu Asp Pro Glu Glu Thr
                             1               5

cag ccg ctt ctg ggg cct cct ggc ggc agc gcg ccc cgc ggc cgc cgc     101
Gln Pro Leu Leu Gly Pro Pro Gly Gly Ser Ala Pro Arg Gly Arg Arg
10                  15                  20                  25

gtc ttc ctc gcc gcc ttc gcc gct gcc ctg ggc cca ctc agc ttc ggc     149
Val Phe Leu Ala Ala Phe Ala Ala Ala Leu Gly Pro Leu Ser Phe Gly
                30                  35                  40

ttc gcg ctc ggc tac agc tcc ccg gcc atc cct agc ctg cag cgc gcc     197
Phe Ala Leu Gly Tyr Ser Ser Pro Ala Ile Pro Ser Leu Gln Arg Ala
            45                  50                  55

gcg ccc ccg gcc ccg cgc ctg gac gac gcc gcc gcc tcc tgg ttc ggg     245
Ala Pro Pro Ala Pro Arg Leu Asp Asp Ala Ala Ala Ser Trp Phe Gly
        60                  65                  70

gct gtc gtg acc ctg ggt gcc gcg gcg ggg gga gtg ctg ggc ggc tgg     293
Ala Val Val Thr Leu Gly Ala Ala Ala Gly Gly Val Leu Gly Gly Trp
    75                  80                  85

ctg gtg gac cgc gcc ggg cgc aag ctg agc ctc ttg ctg tgc tcc gtg     341
Leu Val Asp Arg Ala Gly Arg Lys Leu Ser Leu Leu Leu Cys Ser Val
90                  95                  100                 105

ccc ttc gtg gcc ggc ttt gcc gtc atc acc gcg gcc cag gac gtg tgg     389
Pro Phe Val Ala Gly Phe Ala Val Ile Thr Ala Ala Gln Asp Val Trp
                110                 115                 120

atg ctg ctg ggg ggc cgc ctc ctc acc ggc ctg gcc tgc ggt gtt gcc     437
Met Leu Leu Gly Gly Arg Leu Leu Thr Gly Leu Ala Cys Gly Val Ala
            125                 130                 135

tcc cta gtg gcc ccg gtc tac atc tcc gaa atc gcc tac cca gca gtc     485
Ser Leu Val Ala Pro Val Tyr Ile Ser Glu Ile Ala Tyr Pro Ala Val
        140                 145                 150

cgg ggg ttg ctc ggc tcc tgt gtg cag cta atg gtc gtc gtc ggc atc     533
Arg Gly Leu Leu Gly Ser Cys Val Gln Leu Met Val Val Val Gly Ile
    155                 160                 165

ctc ctg gcc tac ctg gca ggc tgg gtg ctg gag tgg cgc tgg ctg gct     581
Leu Leu Ala Tyr Leu Ala Gly Trp Val Leu Glu Trp Arg Trp Leu Ala
170                 175                 180                 185

gtg ctg ggc tgc gtg ccc ccc tcc ctc atg ctg ctt ctc atg tgc ttc     629
Val Leu Gly Cys Val Pro Pro Ser Leu Met Leu Leu Leu Met Cys Phe
                190                 195                 200

atg ccc gag acc ccg cgc ttc ctg ctg act cag cac agg cgc cag gag     677
Met Pro Glu Thr Pro Arg Phe Leu Leu Thr Gln His Arg Arg Gln Glu
            205                 210                 215

gcc atg gcc gcc ctg cgg ttc ctg tgg ggc tcc gag cag ggc tgg gaa     725
Ala Met Ala Ala Leu Arg Phe Leu Trp Gly Ser Glu Gln Gly Trp Glu
```

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        220                 225                 230

gac ccc ccc atc ggg gct gag cag agc ttt cac ctg gcc ctg ctg cgg      773
Asp Pro Pro Ile Gly Ala Glu Gln Ser Phe His Leu Ala Leu Leu Arg
    235                 240                 245

cag ccc ggc atc tac aag ccc ttc atc atc ggc gtc tcc ctg atg gcc      821
Gln Pro Gly Ile Tyr Lys Pro Phe Ile Ile Gly Val Ser Leu Met Ala
250                 255                 260                 265

ttc cag cag ctg tcg ggg gtc aac gcc gtc atg ttc tat gca gag acc      869
Phe Gln Gln Leu Ser Gly Val Asn Ala Val Met Phe Tyr Ala Glu Thr
                270                 275                 280

atc ttt gaa gag gcc aag ttc aag gac agc agc ctg gcc tcg gtc gtc      917
Ile Phe Glu Glu Ala Lys Phe Lys Asp Ser Ser Leu Ala Ser Val Val
            285                 290                 295

gtg ggt gtc atc cag gtg ctg ttc aca gct gtg gcg gct ctc atc atg      965
Val Gly Val Ile Gln Val Leu Phe Thr Ala Val Ala Ala Leu Ile Met
        300                 305                 310

gac aga gca ggg cgg agg ctg ctc ctg gtc ttg tca ggt gtg gtc atg     1013
Asp Arg Ala Gly Arg Arg Leu Leu Leu Val Leu Ser Gly Val Val Met
    315                 320                 325

gtg ttc agc acg agt gcc ttc ggc gcc tac ttc aag ctg acc cag ggt     1061
Val Phe Ser Thr Ser Ala Phe Gly Ala Tyr Phe Lys Leu Thr Gln Gly
330                 335                 340                 345

ggc cct ggc aac tcc tcg cac gtg gcc atc tcg gcg cct gtc tct gca     1109
Gly Pro Gly Asn Ser Ser His Val Ala Ile Ser Ala Pro Val Ser Ala
                350                 355                 360

cag cct gtt gat gcc agc gtg ggg ctg gcc tgg ctg gcc gtg ggc agc     1157
Gln Pro Val Asp Ala Ser Val Gly Leu Ala Trp Leu Ala Val Gly Ser
            365                 370                 375

atg tgc ctc ttc atc gcc ggc ttt gcg gtg ggc tgg ggg ccc atc ccc     1205
Met Cys Leu Phe Ile Ala Gly Phe Ala Val Gly Trp Gly Pro Ile Pro
        380                 385                 390

tgg ctc ctc atg tca gag atc ttc cct ctg cat gtc aag ggc gtg gcg     1253
Trp Leu Leu Met Ser Glu Ile Phe Pro Leu His Val Lys Gly Val Ala
    395                 400                 405

aca ggc atc tgc gtc ctc acc aac tgg ctc atg gcc ttt ctc gtg acc     1301
Thr Gly Ile Cys Val Leu Thr Asn Trp Leu Met Ala Phe Leu Val Thr
410                 415                 420                 425

aag gag ttc agc agc ctc atg gag gtc ctc agg ccc tat gga gcc ttc     1349
Lys Glu Phe Ser Ser Leu Met Glu Val Leu Arg Pro Tyr Gly Ala Phe
                430                 435                 440

tgg ctt gcc tcc gct ttc tgc atc ttc agt gtc ctt ttc act ttt tcc     1397
Trp Leu Ala Ser Ala Phe Cys Ile Phe Ser Val Leu Phe Thr Phe Ser
            445                 450                 455

tgt gtc cct gaa act aaa gga aag act ctg gaa caa atc aca gcc cat     1445
Cys Val Pro Glu Thr Lys Gly Lys Thr Leu Glu Gln Ile Thr Ala His
        460                 465                 470

ttt gag ggg cga tga cagccactca ctaggggatg gagcaagcct gtgactccaa     1500
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Phe Glu Gly Arg
475

gctgggcc 1508

<210> 48
<211> 477
<212> PRT
<213> GLUT8

<400> 48

Met Thr Pro Glu Asp Pro Glu Glu Thr Gln Pro Leu Leu Gly Pro Pro
1 5 10 15

Gly Gly Ser Ala Pro Arg Gly Arg Arg Val Phe Leu Ala Ala Phe Ala
20 25 30

Ala Ala Leu Gly Pro Leu Ser Phe Gly Phe Ala Leu Gly Tyr Ser Ser
35 40 45

Pro Ala Ile Pro Ser Leu Gln Arg Ala Ala Pro Pro Ala Pro Arg Leu
50 55 60

Asp Asp Ala Ala Ala Ser Trp Phe Gly Ala Val Val Thr Leu Gly Ala
65 70 75 80

Ala Ala Gly Gly Val Leu Gly Gly Trp Leu Val Asp Arg Ala Gly Arg
85 90 95

Lys Leu Ser Leu Leu Leu Cys Ser Val Pro Phe Val Ala Gly Phe Ala
100 105 110

Val Ile Thr Ala Ala Gln Asp Val Trp Met Leu Leu Gly Gly Arg Leu
115 120 125

Leu Thr Gly Leu Ala Cys Gly Val Ala Ser Leu Val Ala Pro Val Tyr
130 135 140

Ile Ser Glu Ile Ala Tyr Pro Ala Val Arg Gly Leu Leu Gly Ser Cys
145 150 155 160

Val Gln Leu Met Val Val Val Gly Ile Leu Leu Ala Tyr Leu Ala Gly
165 170 175

Trp Val Leu Glu Trp Arg Trp Leu Ala Val Leu Gly Cys Val Pro Pro
180 185 190

Ser Leu Met Leu Leu Leu Met Cys Phe Met Pro Glu Thr Pro Arg Phe
195 200 205

Leu Leu Thr Gln His Arg Arg Gln Glu Ala Met Ala Ala Leu Arg Phe
210 215 220

Leu Trp Gly Ser Glu Gln Gly Trp Glu Asp Pro Pro Ile Gly Ala Glu
225 230 235 240

Gln Ser Phe His Leu Ala Leu Leu Arg Gln Pro Gly Ile Tyr Lys Pro
245 250 255

Phe Ile Ile Gly Val Ser Leu Met Ala Phe Gln Gln Leu Ser Gly Val
260 265 270

Asn Ala Val Met Phe Tyr Ala Glu Thr Ile Phe Glu Glu Ala Lys Phe
275 280 285

Lys Asp Ser Ser Leu Ala Ser Val Val Val Gly Val Ile Gln Val Leu
290 295 300

Phe Thr Ala Val Ala Ala Leu Ile Met Asp Arg Ala Gly Arg Arg Leu
305 310 315 320

Leu Leu Val Leu Ser Gly Val Val Met Val Phe Ser Thr Ser Ala Phe
325 330 335

Gly Ala Tyr Phe Lys Leu Thr Gln Gly Gly Pro Gly Asn Ser Ser His
340 345 350

Val Ala Ile Ser Ala Pro Val Ser Ala Gln Pro Val Asp Ala Ser Val
355 360 365

Gly Leu Ala Trp Leu Ala Val Gly Ser Met Cys Leu Phe Ile Ala Gly
370 375 380

Phe Ala Val Gly Trp Gly Pro Ile Pro Trp Leu Leu Met Ser Glu Ile
385 390 395 400

Phe Pro Leu His Val Lys Gly Val Ala Thr Gly Ile Cys Val Leu Thr
405 410 415

Asn Trp Leu Met Ala Phe Leu Val Thr Lys Glu Phe Ser Ser Leu Met
420 425 430

Glu Val Leu Arg Pro Tyr Gly Ala Phe Trp Leu Ala Ser Ala Phe Cys
435 440 445

Ile Phe Ser Val Leu Phe Thr Phe Ser Cys Val Pro Glu Thr Lys Gly

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    450                 455                 460

Lys Thr Leu Glu Gln Ile Thr Ala His Phe Glu Gly Arg
465                 470                 475


<210>  49
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<212>  DNA
<213>  GLUT9

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<221>  CDS
<222>  (55)..(1677)
<223>

<400>  49
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                                                           Met
                                                           1

gca agg aaa caa aat agg aat tcc aag gaa ctg ggc cta gtt ccc ctc      105
Ala Arg Lys Gln Asn Arg Asn Ser Lys Glu Leu Gly Leu Val Pro Leu
            5                   10                  15

aca gat gac acc agc cac gcc agg cct cca ggg cca ggg agg gca ctg      153
Thr Asp Asp Thr Ser His Ala Arg Pro Pro Gly Pro Gly Arg Ala Leu
        20                  25                  30

ctg gag tgt gac cac ctg agg agt ggg gtg cca ggt gga agg aga aga      201
Leu Glu Cys Asp His Leu Arg Ser Gly Val Pro Gly Gly Arg Arg Arg
    35                  40                  45

aag gac tgg tcc tgc tcg ctc ctc gtg gcc tcc ctc gcg ggc gcc ttc      249
Lys Asp Trp Ser Cys Ser Leu Leu Val Ala Ser Leu Ala Gly Ala Phe
50                  55                  60                  65

ggc tcc tcc ttc ctc tac ggc tac aac ctg tcg gtg gtg aat gcc ccc      297
Gly Ser Ser Phe Leu Tyr Gly Tyr Asn Leu Ser Val Val Asn Ala Pro
                70                  75                  80

acc ccg tac atc aag gcc ttt tac aat gag tca tgg gaa aga agg cat      345
Thr Pro Tyr Ile Lys Ala Phe Tyr Asn Glu Ser Trp Glu Arg Arg His
            85                  90                  95

gga cgt cca ata gac cca gac act ctg act ctg ctc tgg tct gtg act      393
Gly Arg Pro Ile Asp Pro Asp Thr Leu Thr Leu Leu Trp Ser Val Thr
        100                 105                 110

gtg tcc ata ttc gcc atc ggt gga ctt gtg ggg aca tta att gtg aag      441
Val Ser Ile Phe Ala Ile Gly Gly Leu Val Gly Thr Leu Ile Val Lys
    115                 120                 125

atg att gga aag gtt ctt ggg agg aag cac act ttg ctg gcc aat aat      489
Met Ile Gly Lys Val Leu Gly Arg Lys His Thr Leu Leu Ala Asn Asn
130                 135                 140                 145

ggg ttt gca att tct gct gca ttg ctg atg gcc tgc tcg ctc cag gca      537
Gly Phe Ala Ile Ser Ala Ala Leu Leu Met Ala Cys Ser Leu Gln Ala
                150                 155                 160

gga gcc ttt gaa atg ctc atc gtg gga cgc ttc atc atg ggc ata gat      585
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Gly Ala Phe Glu Met Leu Ile Val Gly Arg Phe Ile Met Gly Ile Asp
           165                 170                 175

gga ggc gtc gcc ctc agt gtg ctc ccc atg tac ctc agt gag atc tca      633
Gly Gly Val Ala Leu Ser Val Leu Pro Met Tyr Leu Ser Glu Ile Ser
        180                 185                 190

ccc aag gag atc cgt ggc tct ctg ggg cag gtg act gcc atc ttt atc      681
Pro Lys Glu Ile Arg Gly Ser Leu Gly Gln Val Thr Ala Ile Phe Ile
    195                 200                 205

tgc att ggc gtg ttc act ggg cag ctt ctg ggc ctg ccc gag ctg ctg      729
Cys Ile Gly Val Phe Thr Gly Gln Leu Leu Gly Leu Pro Glu Leu Leu
210                 215                 220                 225

gga aag gag agt acc tgg cca tac ctg ttt gga gtg att gtg gtc cct      777
Gly Lys Glu Ser Thr Trp Pro Tyr Leu Phe Gly Val Ile Val Val Pro
                230                 235                 240

gcc gtt gtc cag ctg ctg agc ctt ccc ttt ctc ccg gac agc cca cgc      825
Ala Val Val Gln Leu Leu Ser Leu Pro Phe Leu Pro Asp Ser Pro Arg
            245                 250                 255

tac ctg ctc ttg gag aag cac aac gag gca aga gct gtg aaa gcc ttc      873
Tyr Leu Leu Leu Glu Lys His Asn Glu Ala Arg Ala Val Lys Ala Phe
        260                 265                 270

caa acg ttc ttg ggt aaa gca gac gtt tcc caa gag gta gag gag gtc      921
Gln Thr Phe Leu Gly Lys Ala Asp Val Ser Gln Glu Val Glu Glu Val
    275                 280                 285

ctg gct gag agc cgc gtg cag agg agc atc cgc ctg gtg tcc gtg ctg      969
Leu Ala Glu Ser Arg Val Gln Arg Ser Ile Arg Leu Val Ser Val Leu
290                 295                 300                 305

gag ctg ctg aga gct ccc tac gtc cgc tgg cag gtg gtc acc gtg att     1017
Glu Leu Leu Arg Ala Pro Tyr Val Arg Trp Gln Val Val Thr Val Ile
                310                 315                 320

gtc acc atg gcc tgc tac cag ctc tgt ggc ctc aat gca att tgg ttc     1065
Val Thr Met Ala Cys Tyr Gln Leu Cys Gly Leu Asn Ala Ile Trp Phe
            325                 330                 335

tat acc aac agc atc ttt gga aaa gct ggg atc cct ctg gca aag atc     1113
Tyr Thr Asn Ser Ile Phe Gly Lys Ala Gly Ile Pro Leu Ala Lys Ile
        340                 345                 350

cca tac gtc acc ttg agt aca ggg ggc atc gag act ttg gct gcc gtc     1161
Pro Tyr Val Thr Leu Ser Thr Gly Gly Ile Glu Thr Leu Ala Ala Val
    355                 360                 365

ttc tct ggt ttg gtc att gag cac ctg gga cgg aga ccc ctc ctc att     1209
Phe Ser Gly Leu Val Ile Glu His Leu Gly Arg Arg Pro Leu Leu Ile
370                 375                 380                 385

ggt ggc ttt ggg ctc atg ggc ctc ttc ttt ggg acc ctc acc atc acg     1257
Gly Gly Phe Gly Leu Met Gly Leu Phe Phe Gly Thr Leu Thr Ile Thr
                390                 395                 400

ctg acc ctg cag gac cac gcc ccc tgg gtc ccc tac ctg agt atc gtg     1305
Leu Thr Leu Gln Asp His Ala Pro Trp Val Pro Tyr Leu Ser Ile Val
            405                 410                 415
```

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ggc att ctg gcc atc atc gcc tct ttc tgc agt ggg cca ggt ggc atc     1353
Gly Ile Leu Ala Ile Ile Ala Ser Phe Cys Ser Gly Pro Gly Gly Ile
        420                 425                 430

ccg ttc atc ttg act ggt gag ttc ttc cag caa tct cag cgg ccg gct     1401
Pro Phe Ile Leu Thr Gly Glu Phe Phe Gln Gln Ser Gln Arg Pro Ala
    435                 440                 445

gcc ttc atc att gca ggc acc gtc aac tgg ctc tcc aac ttt gct gtt     1449
Ala Phe Ile Ile Ala Gly Thr Val Asn Trp Leu Ser Asn Phe Ala Val
450                 455                 460                 465

ggg ctc ctc ttc cca ttc att cag aaa agt ctg gac acc tac tgt ttc     1497
Gly Leu Leu Phe Pro Phe Ile Gln Lys Ser Leu Asp Thr Tyr Cys Phe
                470                 475                 480

cta gtc ttt gct aca att tgt atc aca ggt gct atc tac ctg tat ttt     1545
Leu Val Phe Ala Thr Ile Cys Ile Thr Gly Ala Ile Tyr Leu Tyr Phe
            485                 490                 495

gtg ctg cct gag acc aaa aac aga acc tat gca gaa atc agc cag gca     1593
Val Leu Pro Glu Thr Lys Asn Arg Thr Tyr Ala Glu Ile Ser Gln Ala
        500                 505                 510

ttt tcc aaa agg aac aaa gca tac cca cca gaa gag aaa atc gac tca     1641
Phe Ser Lys Arg Asn Lys Ala Tyr Pro Pro Glu Glu Lys Ile Asp Ser
    515                 520                 525

gct gtc act gat ggt aag ata aat gga agg cct taa caagtttcct          1687
Ala Val Thr Asp Gly Lys Ile Asn Gly Arg Pro
530                 535                 540

cctccacgtt ggacaattat gtcaaaaaca ggattgtcta catggatgat ctcacttttc   1747

aggaaactta aaatttaccc attattggga agcttaaatg aattgaagct atgcaagtct   1807

tttatattat taaatattta aaagtaaacc tgtactaatc taaaaaaaaa aaaaaa       1863
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<210> 50
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<212> PRT
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<400> 50

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1               5                   10                  15

Leu Thr Asp Asp Thr Ser His Ala Arg Pro Pro Gly Pro Gly Arg Ala
            20                  25                  30

Leu Leu Glu Cys Asp His Leu Arg Ser Gly Val Pro Gly Gly Arg Arg
        35                  40                  45

Arg Lys Asp Trp Ser Cys Ser Leu Leu Val Ala Ser Leu Ala Gly Ala
    50                  55                  60

Phe Gly Ser Ser Phe Leu Tyr Gly Tyr Asn Leu Ser Val Val Asn Ala
```

65 70 75 80

Pro Thr Pro Tyr Ile Lys Ala Phe Tyr Asn Glu Ser Trp Glu Arg Arg
85 90 95

His Gly Arg Pro Ile Asp Pro Asp Thr Leu Thr Leu Leu Trp Ser Val
100 105 110

Thr Val Ser Ile Phe Ala Ile Gly Gly Leu Val Gly Thr Leu Ile Val
115 120 125

Lys Met Ile Gly Lys Val Leu Gly Arg Lys His Thr Leu Leu Ala Asn
130 135 140

Asn Gly Phe Ala Ile Ser Ala Ala Leu Leu Met Ala Cys Ser Leu Gln
145 150 155 160

Ala Gly Ala Phe Glu Met Leu Ile Val Gly Arg Phe Ile Met Gly Ile
165 170 175

Asp Gly Gly Val Ala Leu Ser Val Leu Pro Met Tyr Leu Ser Glu Ile
180 185 190

Ser Pro Lys Glu Ile Arg Gly Ser Leu Gly Gln Val Thr Ala Ile Phe
195 200 205

Ile Cys Ile Gly Val Phe Thr Gly Gln Leu Leu Gly Leu Pro Glu Leu
210 215 220

Leu Gly Lys Glu Ser Thr Trp Pro Tyr Leu Phe Gly Val Ile Val Val
225 230 235 240

Pro Ala Val Val Gln Leu Leu Ser Leu Pro Phe Leu Pro Asp Ser Pro
245 250 255

Arg Tyr Leu Leu Leu Glu Lys His Asn Glu Ala Arg Ala Val Lys Ala
260 265 270

Phe Gln Thr Phe Leu Gly Lys Ala Asp Val Ser Gln Glu Val Glu Glu
275 280 285

Val Leu Ala Glu Ser Arg Val Gln Arg Ser Ile Arg Leu Val Ser Val
290 295 300

Leu Glu Leu Leu Arg Ala Pro Tyr Val Arg Trp Gln Val Val Thr Val
305 310 315 320

```
Ile Val Thr Met Ala Cys Tyr Gln Leu Cys Gly Leu Asn Ala Ile Trp
                325                 330                 335

Phe Tyr Thr Asn Ser Ile Phe Gly Lys Ala Gly Ile Pro Leu Ala Lys
            340                 345                 350

Ile Pro Tyr Val Thr Leu Ser Thr Gly Gly Ile Glu Thr Leu Ala Ala
        355                 360                 365

Val Phe Ser Gly Leu Val Ile Glu His Leu Gly Arg Arg Pro Leu Leu
    370                 375                 380

Ile Gly Gly Phe Gly Leu Met Gly Leu Phe Phe Gly Thr Leu Thr Ile
385                 390                 395                 400

Thr Leu Thr Leu Gln Asp His Ala Pro Trp Val Pro Tyr Leu Ser Ile
                405                 410                 415

Val Gly Ile Leu Ala Ile Ile Ala Ser Phe Cys Ser Gly Pro Gly Gly
            420                 425                 430

Ile Pro Phe Ile Leu Thr Gly Glu Phe Phe Gln Gln Ser Gln Arg Pro
        435                 440                 445

Ala Ala Phe Ile Ile Ala Gly Thr Val Asn Trp Leu Ser Asn Phe Ala
    450                 455                 460

Val Gly Leu Leu Phe Pro Phe Ile Gln Lys Ser Leu Asp Thr Tyr Cys
465                 470                 475                 480

Phe Leu Val Phe Ala Thr Ile Cys Ile Thr Gly Ala Ile Tyr Leu Tyr
                485                 490                 495

Phe Val Leu Pro Glu Thr Lys Asn Arg Thr Tyr Ala Glu Ile Ser Gln
            500                 505                 510

Ala Phe Ser Lys Arg Asn Lys Ala Tyr Pro Pro Glu Glu Lys Ile Asp
        515                 520                 525

Ser Ala Val Thr Asp Gly Lys Ile Asn Gly Arg Pro
    530                 535                 540
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<213> GLUT10

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<221> CDS

<222> (53)..(1678)
<223>

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Met Gly
1

cac tcc cca cct gtc ctg cct ttg tgt gcc tct gtg tct ttg ctg ggt 106
His Ser Pro Pro Val Leu Pro Leu Cys Ala Ser Val Ser Leu Leu Gly
5 10 15

ggc ctg acc ttt ggt tat gaa ctg gca gtc ata tca ggt gcc ctg ctg 154
Gly Leu Thr Phe Gly Tyr Glu Leu Ala Val Ile Ser Gly Ala Leu Leu
20 25 30

cca ctg cag ctt gac ttt ggg cta agc tgc ttg gag cag gag ttc ctg 202
Pro Leu Gln Leu Asp Phe Gly Leu Ser Cys Leu Glu Gln Glu Phe Leu
35 40 45 50

gtg ggc agc ctg ctc ctg ggg gct ctc ctc gcc tcc ctg gtt ggt ggc 250
Val Gly Ser Leu Leu Leu Gly Ala Leu Leu Ala Ser Leu Val Gly Gly
55 60 65

ttc ctc att gac tgc tat ggc agg aag caa gcc atc ctc ggg agc aac 298
Phe Leu Ile Asp Cys Tyr Gly Arg Lys Gln Ala Ile Leu Gly Ser Asn
70 75 80

ttg gtg ctg ctg gca ggc agc ctg acc ctg ggc ctg gct ggt tcc ctg 346
Leu Val Leu Leu Ala Gly Ser Leu Thr Leu Gly Leu Ala Gly Ser Leu
85 90 95

gcc tgg ctg gtc ctg ggc cgc gct gtg gtt ggc ttc gcc att tcc ctc 394
Ala Trp Leu Val Leu Gly Arg Ala Val Val Gly Phe Ala Ile Ser Leu
100 105 110

tcc tcc atg gct tgc tgt atc tac gtg tca gag ctg gtg ggg cca cgg 442
Ser Ser Met Ala Cys Cys Ile Tyr Val Ser Glu Leu Val Gly Pro Arg
115 120 125 130

cag cgg gga gtg ctg gtg tcc ctc tat gag gca ggc atc acc gtg ggc 490
Gln Arg Gly Val Leu Val Ser Leu Tyr Glu Ala Gly Ile Thr Val Gly
135 140 145

atc ctg ctc tcc tat gcc ctc aac tat gca ctg gct ggt acc ccc tgg 538
Ile Leu Leu Ser Tyr Ala Leu Asn Tyr Ala Leu Ala Gly Thr Pro Trp
150 155 160

gga tgg agg cac atg ttc ggc tgg gcc act gca cct gct gtc ctg caa 586
Gly Trp Arg His Met Phe Gly Trp Ala Thr Ala Pro Ala Val Leu Gln
165 170 175

tcc ctc agc ctc ctc ttc ctc cct gct ggt aca gat gag act gca aca 634
Ser Leu Ser Leu Leu Phe Leu Pro Ala Gly Thr Asp Glu Thr Ala Thr
180 185 190

cac aag gac ctc atc cca ctc cag gga ggt gag gcc ccc aag ctg ggc 682
His Lys Asp Leu Ile Pro Leu Gln Gly Gly Glu Ala Pro Lys Leu Gly
195 200 205 210

ccg ggg agg cca cgg tac tcc ttt ctg gac ctc ttc agg gca cgc gat 730
Pro Gly Arg Pro Arg Tyr Ser Phe Leu Asp Leu Phe Arg Ala Arg Asp
215 220 225

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aac atg cga ggc cgg acc aca gtg ggc ctg ggg ctg gtg ctc ttc cag      778
Asn Met Arg Gly Arg Thr Thr Val Gly Leu Gly Leu Val Leu Phe Gln
            230                 235                 240

caa cta aca ggg cag ccc aac gtg ctg tgc tat gcc tcc acc atc ttc      826
Gln Leu Thr Gly Gln Pro Asn Val Leu Cys Tyr Ala Ser Thr Ile Phe
        245                 250                 255

agc tcc gtt ggt ttc cat ggg gga tcc tca gcc gtg ctg gcc tct gtg      874
Ser Ser Val Gly Phe His Gly Gly Ser Ser Ala Val Leu Ala Ser Val
    260                 265                 270

ggg ctt ggc gca gtg aag gtg gca gct acc ctg acc gcc atg ggg ctg      922
Gly Leu Gly Ala Val Lys Val Ala Ala Thr Leu Thr Ala Met Gly Leu
275                 280                 285                 290

gtg gac cgt gca ggc cgc agg gct ctg ttg cta gct ggc tgt gcc ctc      970
Val Asp Arg Ala Gly Arg Arg Ala Leu Leu Leu Ala Gly Cys Ala Leu
                295                 300                 305

atg gcc ctg tcc gtc agt ggc ata ggc ctc gtc agc ttt gcc gtg ccc     1018
Met Ala Leu Ser Val Ser Gly Ile Gly Leu Val Ser Phe Ala Val Pro
            310                 315                 320

atg gac tca ggc cca agc tgt ctg gct gtg ccc aat gcc acc ggg cag     1066
Met Asp Ser Gly Pro Ser Cys Leu Ala Val Pro Asn Ala Thr Gly Gln
        325                 330                 335

aca ggc ctc cct gga gac tct ggc ctg ctg cag gac tcc tct cta cct     1114
Thr Gly Leu Pro Gly Asp Ser Gly Leu Leu Gln Asp Ser Ser Leu Pro
    340                 345                 350

ccc att cca agg acc aat gag gac caa agg gag cca atc ttg tcc act     1162
Pro Ile Pro Arg Thr Asn Glu Asp Gln Arg Glu Pro Ile Leu Ser Thr
355                 360                 365                 370

gct aag aaa acc aag ccc cat ccc aga tct gga gac ccc tca gcc cct     1210
Ala Lys Lys Thr Lys Pro His Pro Arg Ser Gly Asp Pro Ser Ala Pro
                375                 380                 385

cct cgg ctg gcc ctg agc tct gcc ctc cct ggg ccc cct ctg ccc gct     1258
Pro Arg Leu Ala Leu Ser Ser Ala Leu Pro Gly Pro Pro Leu Pro Ala
            390                 395                 400

cgg ggg cat gca ctg ctg cgc tgg acc gca ctg ctg tgc ctg atg gtc     1306
Arg Gly His Ala Leu Leu Arg Trp Thr Ala Leu Leu Cys Leu Met Val
        405                 410                 415

ttt gtc agt gcc ttc tcc ttt ggg ttt ggg cca gtg acc tgg ctt gtc     1354
Phe Val Ser Ala Phe Ser Phe Gly Phe Gly Pro Val Thr Trp Leu Val
    420                 425                 430

ctc agc gag atc tac cct gtg gag ata cga gga aga gcc ttc gcc ttc     1402
Leu Ser Glu Ile Tyr Pro Val Glu Ile Arg Gly Arg Ala Phe Ala Phe
435                 440                 445                 450

tgc aac agc ttc aac tgg gcg gcc aac ctc ttc atc agc ctc tcc ttc     1450
Cys Asn Ser Phe Asn Trp Ala Ala Asn Leu Phe Ile Ser Leu Ser Phe
                455                 460                 465

ctc gat ctc att ggc acc atc ggc ttg tcc tgg acc ttc ctg ctc tac     1498
Leu Asp Leu Ile Gly Thr Ile Gly Leu Ser Trp Thr Phe Leu Leu Tyr
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            470                 475                 480

gga ctg acc gct gtc ctc ggc ctg ggc ttc atc tat tta ttt gtt cct     1546
Gly Leu Thr Ala Val Leu Gly Leu Gly Phe Ile Tyr Leu Phe Val Pro
        485                 490                 495

gaa aca aaa ggc cag tcg ttg gca gag ata gac cag cag ttc cag aag     1594
Glu Thr Lys Gly Gln Ser Leu Ala Glu Ile Asp Gln Gln Phe Gln Lys
    500                 505                 510

aga cgg ttc acc ctg agc ttt ggc cac agg cag aac tcc act ggc atc     1642
Arg Arg Phe Thr Leu Ser Phe Gly His Arg Gln Asn Ser Thr Gly Ile
515                 520                 525                 530

ccg tac agc cgc atc gag atc tct gcg gcc tcc tga ggaatccgtc          1688
Pro Tyr Ser Arg Ile Glu Ile Ser Ala Ala Ser
                535                 540

tgcctggaaa ttctggaact gtggctttgg cagaccatct ccagcatcct gcttcctagg   1748

ccccagagca caagttccag ctggtctttt gggagtggcc cctgccccca aaggtggtct   1808

gcttttgctg gggtaaaaag gatgaaagtc tgagaatgcc caactcttca ttttgagtct   1868

caggccctga aggttcctga ggatctagct tcatgcctca gtttccccat tgacttgcac   1928

atctctgcag tatttataag aagaatattc tatgaagtct ttgttgcacc atggactttt   1988

ctcaaagaat ctcaagggta ccaatcctgg caggaagtct ctcccgatat cacccctaaa   2048

tccaaatgag gatatcatct tttctaatct ctttttttcaa ctggctggga cattttcgga   2108

agggggaagt ctctttttttt actcttatca ttttttttttt ttgaggtgga gtctcattct   2168

gttgcccagg ctggcctgat cttggctcac tgcaacctcc acctcctgag ttcaagcgat   2228

tcttgtgcct cagcctccta agcagctggg actacaggcg catgcaacca tacccagcta   2288

atttatttttt agcagagatg gggtttcact gtgttggcca ggctggtcgt gaactcctga   2348

gctcaagtga tccacccacc tcagcctccc agagtgctag gattacaggc cttttgactc   2408

ttttatctga gttttattga cccctctaat tctcttaccc agaatattta tccttcacca   2468

gcaactctga ctctttgacg ggaggcctca gttctagtcc ttggtctgct ggtgtcattg   2528

ctgtaggaat gaccacgggc ctcagtttcc ccatttgtat aatgggaagc ctgtaccagg   2588

tcattcttaa gatttctcct gactccagtg agctggaatt ctaaatgctg gtctaggagc   2648

tgtctccagg atggtgcagg atggctttgc ggaaaggaga tgggtttgga ggccaacaaa   2708

cctgcttgtc aatattgcct ttgcctcttg gcagcccttg aacttgagta aataacaact   2768

ccctgaacct cagtttcctc atctgcagaa tggggataat tatgtcccag gggtatattt   2828

agaccctgtt tcctttcagg agggtcccca gctggtccag ggcctgggaa atttctactt   2888

atcctcatta cccaggtccc tcctttggac cctgtaaagg gtcagggtga atcagatggg   2948

ggactgagca agtagctatg actgcagatc atgtaaggaa gggactgaca agaagctccc   3008

agatgctggg gagaatgaag agctaaaata gatcctaggt gctggatgct ttgtcatcca   3068
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tgcgtgcaca tatgggtgct ggcagagccc ccaaggactc tggcctctcg agttctccta 3128

tcttctccat tctagatgct tcccttgtat ccagtgatgt gctggagctg gctttgccaa 3188

gcttgtgaga gctggttgct acattttcag gatttttaca agttggtaaa cacagccatt 3248

ataaaaaatt aaatgattta aatttataat taagtaaatt acattaaaac aaaaaaatta 3308

tactcaaaat tcattactta attttactac ctgttactat tatctgtgct tttgaggcta 3368

tttctacata gtaactctta tggagaccta ggggagacac cgcgcatctc ttcctgattc 3428

cccactcaat gacatcatgt tagtctttgg ttgcttaact ggctgtgggg agtgtttttg 3488

tatcacaaag attagagagg actacacatc agggcttgat ttattgtttg ttgattttct 3548

agacttcaga acatgctgga taaaatgtca gtaatgcaaa ttaaacttta aagtatgtct 3608

tgtttgtagc caatacatgg tgtatagcac caaaaaatgg agggattatt cttccagtag 3668

ttgaacactg tcatccgttt cagctgacag ctgctcaaat catttaagaa ggagttctga 3728

cattcatttt cattgtttta cttttgtctt cctcactagt gtaaacaaaa atttcaacca 3788

gcattcatgc cgaacctata cccattcttc agtgcctagc tgtacagtta tcagggattt 3848

ttatttgtag tctaattttg tcaaatcatg gccaaatcgc agtgatagtt gactttggat 3908

acaaggtttg gcaaaaaaaa aaatattaac aaaatattct gtaagaatca attgtctata 3968

tggaatttag gataaagaat atttacaata aagaatattt acaataaaga gtttattatt 4028

atttgtaagt tgtgtgcaac aaacataccc tttatctctg taaaatttat acacacaaaa 4088

attaacaaaa gattctgtaa gaattaattg gctatatgga atttaggata gaatatttac 4148

aataaagagt atttacaat 4167

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<212> PRT
<213> GLUT10

<400> 52

Met Gly His Ser Pro Pro Val Leu Pro Leu Cys Ala Ser Val Ser Leu
1 5 10 15

Leu Gly Gly Leu Thr Phe Gly Tyr Glu Leu Ala Val Ile Ser Gly Ala
20 25 30

Leu Leu Pro Leu Gln Leu Asp Phe Gly Leu Ser Cys Leu Glu Gln Glu
35 40 45

Phe Leu Val Gly Ser Leu Leu Leu Gly Ala Leu Leu Ala Ser Leu Val
50 55 60

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Gly Gly Phe Leu Ile Asp Cys Tyr Gly Arg Lys Gln Ala Ile Leu Gly
65                  70                  75                  80

Ser Asn Leu Val Leu Leu Ala Gly Ser Leu Thr Leu Gly Leu Ala Gly
                85                  90                  95

Ser Leu Ala Trp Leu Val Leu Gly Arg Ala Val Val Gly Phe Ala Ile
            100                 105                 110

Ser Leu Ser Ser Met Ala Cys Cys Ile Tyr Val Ser Glu Leu Val Gly
        115                 120                 125

Pro Arg Gln Arg Gly Val Leu Val Ser Leu Tyr Glu Ala Gly Ile Thr
    130                 135                 140

Val Gly Ile Leu Leu Ser Tyr Ala Leu Asn Tyr Ala Leu Ala Gly Thr
145                 150                 155                 160

Pro Trp Gly Trp Arg His Met Phe Gly Trp Ala Thr Ala Pro Ala Val
                165                 170                 175

Leu Gln Ser Leu Ser Leu Leu Phe Leu Pro Ala Gly Thr Asp Glu Thr
            180                 185                 190

Ala Thr His Lys Asp Leu Ile Pro Leu Gln Gly Gly Glu Ala Pro Lys
        195                 200                 205

Leu Gly Pro Gly Arg Pro Arg Tyr Ser Phe Leu Asp Leu Phe Arg Ala
    210                 215                 220

Arg Asp Asn Met Arg Gly Arg Thr Thr Val Gly Leu Gly Leu Val Leu
225                 230                 235                 240

Phe Gln Gln Leu Thr Gly Gln Pro Asn Val Leu Cys Tyr Ala Ser Thr
                245                 250                 255

Ile Phe Ser Ser Val Gly Phe His Gly Gly Ser Ser Ala Val Leu Ala
            260                 265                 270

Ser Val Gly Leu Gly Ala Val Lys Val Ala Ala Thr Leu Thr Ala Met
        275                 280                 285

Gly Leu Val Asp Arg Ala Gly Arg Arg Ala Leu Leu Leu Ala Gly Cys
    290                 295                 300

Ala Leu Met Ala Leu Ser Val Ser Gly Ile Gly Leu Val Ser Phe Ala
305                 310                 315                 320
```

Val Pro Met Asp Ser Gly Pro Ser Cys Leu Ala Val Pro Asn Ala Thr
325 330 335

Gly Gln Thr Gly Leu Pro Gly Asp Ser Gly Leu Leu Gln Asp Ser Ser
340 345 350

Leu Pro Pro Ile Pro Arg Thr Asn Glu Asp Gln Arg Glu Pro Ile Leu
355 360 365

Ser Thr Ala Lys Lys Thr Lys Pro His Pro Arg Ser Gly Asp Pro Ser
370 375 380

Ala Pro Pro Arg Leu Ala Leu Ser Ser Ala Leu Pro Gly Pro Pro Leu
385 390 395 400

Pro Ala Arg Gly His Ala Leu Leu Arg Trp Thr Ala Leu Leu Cys Leu
405 410 415

Met Val Phe Val Ser Ala Phe Ser Phe Gly Phe Gly Pro Val Thr Trp
420 425 430

Leu Val Leu Ser Glu Ile Tyr Pro Val Glu Ile Arg Gly Arg Ala Phe
435 440 445

Ala Phe Cys Asn Ser Phe Asn Trp Ala Ala Asn Leu Phe Ile Ser Leu
450 455 460

Ser Phe Leu Asp Leu Ile Gly Thr Ile Gly Leu Ser Trp Thr Phe Leu
465 470 475 480

Leu Tyr Gly Leu Thr Ala Val Leu Gly Leu Gly Phe Ile Tyr Leu Phe
485 490 495

Val Pro Glu Thr Lys Gly Gln Ser Leu Ala Glu Ile Asp Gln Gln Phe
500 505 510

Gln Lys Arg Arg Phe Thr Leu Ser Phe Gly His Arg Gln Asn Ser Thr
515 520 525

Gly Ile Pro Tyr Ser Arg Ile Glu Ile Ser Ala Ala Ser
530 535 540

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<213> GLUT11

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<222> (100)..(1590)
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gcattccgcc aagtctctcg ctctgcccag gacgcacag atg aga gcg ctc cga 114
Met Arg Ala Leu Arg
1 5

aga ctg att cag ggc agg atc ctg ctc ctg acc atc tgc gct gcc ggc 162
Arg Leu Ile Gln Gly Arg Ile Leu Leu Leu Thr Ile Cys Ala Ala Gly
10 15 20

att ggt ggg act ttt cag ttt ggc tat aac ctc tct atc atc aat gcc 210
Ile Gly Gly Thr Phe Gln Phe Gly Tyr Asn Leu Ser Ile Ile Asn Ala
25 30 35

ccg acc ttg cac att cag gaa ttc acc aat gag aca tgg cag gcg cgt 258
Pro Thr Leu His Ile Gln Glu Phe Thr Asn Glu Thr Trp Gln Ala Arg
40 45 50

act gga gag cca ctg ccc gat cac cta gtc ctg ctt atg tgg tcc ctc 306
Thr Gly Glu Pro Leu Pro Asp His Leu Val Leu Leu Met Trp Ser Leu
55 60 65

atc gtg tct ctg tat ccc ctg gga ggc ctc ttt gga gca ctg ctt gca 354
Ile Val Ser Leu Tyr Pro Leu Gly Gly Leu Phe Gly Ala Leu Leu Ala
70 75 80 85

ggt ccc ttg gcc atc acg ctg gga agg aag aag tcc ctc ctg gtg aat 402
Gly Pro Leu Ala Ile Thr Leu Gly Arg Lys Lys Ser Leu Leu Val Asn
90 95 100

aac atc ttt gtg gtg tca gca gca atc ctg ttt gga ttc agc cgc aaa 450
Asn Ile Phe Val Val Ser Ala Ala Ile Leu Phe Gly Phe Ser Arg Lys
105 110 115

gca ggc tcc ttt gag atg atc atg ctg gga aga ctg ctc gtg gga gtc 498
Ala Gly Ser Phe Glu Met Ile Met Leu Gly Arg Leu Leu Val Gly Val
120 125 130

aat gca ggt gtg agc atg aac atc cag ccc atg tac ctg ggg gag agc 546
Asn Ala Gly Val Ser Met Asn Ile Gln Pro Met Tyr Leu Gly Glu Ser
135 140 145

gcc cct aag gag ctc cga gga gct gtg gcc atg agc tca gcc atc ttt 594
Ala Pro Lys Glu Leu Arg Gly Ala Val Ala Met Ser Ser Ala Ile Phe
150 155 160 165

acg gct ctg ggg atc gtg atg gga cag gtg gtc gga ctc agg gag ctc 642
Thr Ala Leu Gly Ile Val Met Gly Gln Val Val Gly Leu Arg Glu Leu
170 175 180

cta ggt ggc cct cag gcc tgg ccc ctg ctg ctg gcc agc tgc ctg gtg 690
Leu Gly Gly Pro Gln Ala Trp Pro Leu Leu Leu Ala Ser Cys Leu Val
185 190 195

ccc ggg gcg ctc cag ctc gcc tcc ctg cct ctg ctc cct gaa agc ccg 738
Pro Gly Ala Leu Gln Leu Ala Ser Leu Pro Leu Leu Pro Glu Ser Pro
200 205 210

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cgc tac ctc ctc att gac tgt gga gac acc gag gcc tgc ctg gca gca      786
Arg Tyr Leu Leu Ile Asp Cys Gly Asp Thr Glu Ala Cys Leu Ala Ala
    215                 220                 225

cta cgg cgg ctc cgg ggc tcc ggg gac ttg gca ggg gag ctg gag gag      834
Leu Arg Arg Leu Arg Gly Ser Gly Asp Leu Ala Gly Glu Leu Glu Glu
230                 235                 240                 245

ctg gag gag gag cgc gct gcc tgc cag ggc tgc cgt gcc cgg cgc cca      882
Leu Glu Glu Glu Arg Ala Ala Cys Gln Gly Cys Arg Ala Arg Arg Pro
                250                 255                 260

tgg gag ctg ttc cag cat cgg gcc ctg agg aga cag gtg aca agc ctc      930
Trp Glu Leu Phe Gln His Arg Ala Leu Arg Arg Gln Val Thr Ser Leu
            265                 270                 275

gtg gtt ctg ggc agt gcc atg gag ctc tgc ggg aat gac tcg gtg tac      978
Val Val Leu Gly Ser Ala Met Glu Leu Cys Gly Asn Asp Ser Val Tyr
        280                 285                 290

gcc tac gcc tcc tcc gtg ttc cgg aag gca gga gtg ccg gaa gcg aag     1026
Ala Tyr Ala Ser Ser Val Phe Arg Lys Ala Gly Val Pro Glu Ala Lys
    295                 300                 305

atc cag tac gcg atc atc ggg act ggg agc tgc gag ctg ctc acg gcg     1074
Ile Gln Tyr Ala Ile Ile Gly Thr Gly Ser Cys Glu Leu Leu Thr Ala
310                 315                 320                 325

gtt gtt agt tgt gtg gta atc gag agg gtg ggt cgg cgc gtg ctg ctc     1122
Val Val Ser Cys Val Val Ile Glu Arg Val Gly Arg Arg Val Leu Leu
                330                 335                 340

atc ggt ggg tac agc ctg atg acc tgc tgg ggg agc atc ttc act gtg     1170
Ile Gly Gly Tyr Ser Leu Met Thr Cys Trp Gly Ser Ile Phe Thr Val
            345                 350                 355

gcc ctg tgc ctg cag agc tcc ttc ccc tgg aca ctc tac ctg gcc atg     1218
Ala Leu Cys Leu Gln Ser Ser Phe Pro Trp Thr Leu Tyr Leu Ala Met
        360                 365                 370

gcc tgc atc ttt gcc ttc atc ctc agc ttt ggc att ggc cct gcc gga     1266
Ala Cys Ile Phe Ala Phe Ile Leu Ser Phe Gly Ile Gly Pro Ala Gly
    375                 380                 385

gtg acg ggg atc ctg gcc aca gag ctg ttt gac cag atg gcc agg cct     1314
Val Thr Gly Ile Leu Ala Thr Glu Leu Phe Asp Gln Met Ala Arg Pro
390                 395                 400                 405

gct gcc tgc atg gtc tgc ggg gcg ctc atg tgg atc atg ctc atc ctg     1362
Ala Ala Cys Met Val Cys Gly Ala Leu Met Trp Ile Met Leu Ile Leu
                410                 415                 420

gtc ggc ctg gga ttt ccc ttt atc atg gag gcc ttg tcc cac ttc ctc     1410
Val Gly Leu Gly Phe Pro Phe Ile Met Glu Ala Leu Ser His Phe Leu
            425                 430                 435

tat gtc cct ttc ctt ggt gtc tgt gtc tgt ggg gcc atc tac act ggc     1458
Tyr Val Pro Phe Leu Gly Val Cys Val Cys Gly Ala Ile Tyr Thr Gly
        440                 445                 450

ctg ttc ctt cct gag acc aaa ggc aag acc ttc caa gag atc tcc aag     1506
Leu Phe Leu Pro Glu Thr Lys Gly Lys Thr Phe Gln Glu Ile Ser Lys
    455                 460                 465
```

```
gaa tta cac aga ctc aac ttc ccc agg cgg gcc cag ggc ccc acg tgg      1554
Glu Leu His Arg Leu Asn Phe Pro Arg Arg Ala Gln Gly Pro Thr Trp
470                 475                 480                 485

agg agc ctg gag gtt atc cag tca aca gaa ctc tag tcccaaaggg          1600
Arg Ser Leu Glu Val Ile Gln Ser Thr Glu Leu
                490                 495

gtggccag                                                           1608
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<210> 54
<211> 496
<212> PRT
<213> GLUT11

<400> 54

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Met Arg Ala Leu Arg Arg Leu Ile Gln Gly Arg Ile Leu Leu Leu Thr
1               5                   10                  15

Ile Cys Ala Ala Gly Ile Gly Gly Thr Phe Gln Phe Gly Tyr Asn Leu
            20                  25                  30

Ser Ile Ile Asn Ala Pro Thr Leu His Ile Gln Glu Phe Thr Asn Glu
        35                  40                  45

Thr Trp Gln Ala Arg Thr Gly Glu Pro Leu Pro Asp His Leu Val Leu
    50                  55                  60

Leu Met Trp Ser Leu Ile Val Ser Leu Tyr Pro Leu Gly Gly Leu Phe
65                  70                  75                  80

Gly Ala Leu Leu Ala Gly Pro Leu Ala Ile Thr Leu Gly Arg Lys Lys
                85                  90                  95

Ser Leu Leu Val Asn Asn Ile Phe Val Val Ser Ala Ala Ile Leu Phe
            100                 105                 110

Gly Phe Ser Arg Lys Ala Gly Ser Phe Glu Met Ile Met Leu Gly Arg
        115                 120                 125

Leu Leu Val Gly Val Asn Ala Gly Val Ser Met Asn Ile Gln Pro Met
    130                 135                 140

Tyr Leu Gly Glu Ser Ala Pro Lys Glu Leu Arg Gly Ala Val Ala Met
145                 150                 155                 160

Ser Ser Ala Ile Phe Thr Ala Leu Gly Ile Val Met Gly Gln Val Val
                165                 170                 175
```

```
Gly Leu Arg Glu Leu Leu Gly Gly Pro Gln Ala Trp Pro Leu Leu Leu
            180                 185                 190

Ala Ser Cys Leu Val Pro Gly Ala Leu Gln Leu Ala Ser Leu Pro Leu
        195                 200                 205

Leu Pro Glu Ser Pro Arg Tyr Leu Leu Ile Asp Cys Gly Asp Thr Glu
    210                 215                 220

Ala Cys Leu Ala Ala Leu Arg Arg Leu Arg Gly Ser Gly Asp Leu Ala
225                 230                 235                 240

Gly Glu Leu Glu Glu Leu Glu Glu Glu Arg Ala Ala Cys Gln Gly Cys
                245                 250                 255

Arg Ala Arg Arg Pro Trp Glu Leu Phe Gln His Arg Ala Leu Arg Arg
            260                 265                 270

Gln Val Thr Ser Leu Val Val Leu Gly Ser Ala Met Glu Leu Cys Gly
        275                 280                 285

Asn Asp Ser Val Tyr Ala Tyr Ala Ser Ser Val Phe Arg Lys Ala Gly
    290                 295                 300

Val Pro Glu Ala Lys Ile Gln Tyr Ala Ile Ile Gly Thr Gly Ser Cys
305                 310                 315                 320

Glu Leu Leu Thr Ala Val Val Ser Cys Val Val Ile Glu Arg Val Gly
                325                 330                 335

Arg Arg Val Leu Leu Ile Gly Gly Tyr Ser Leu Met Thr Cys Trp Gly
            340                 345                 350

Ser Ile Phe Thr Val Ala Leu Cys Leu Gln Ser Ser Phe Pro Trp Thr
        355                 360                 365

Leu Tyr Leu Ala Met Ala Cys Ile Phe Ala Phe Ile Leu Ser Phe Gly
    370                 375                 380

Ile Gly Pro Ala Gly Val Thr Gly Ile Leu Ala Thr Glu Leu Phe Asp
385                 390                 395                 400

Gln Met Ala Arg Pro Ala Ala Cys Met Val Cys Gly Ala Leu Met Trp
                405                 410                 415

Ile Met Leu Ile Leu Val Gly Leu Gly Phe Pro Phe Ile Met Glu Ala
            420                 425                 430
```

```
Leu Ser His Phe Leu Tyr Val Pro Phe Leu Gly Val Cys Val Cys Gly
        435                 440                 445

Ala Ile Tyr Thr Gly Leu Phe Leu Pro Glu Thr Lys Gly Lys Thr Phe
    450                 455                 460

Gln Glu Ile Ser Lys Glu Leu His Arg Leu Asn Phe Pro Arg Arg Ala
465                 470                 475                 480

Gln Gly Pro Thr Trp Arg Ser Leu Glu Val Ile Gln Ser Thr Glu Leu
                485                 490                 495
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<210> 55
<211> 2223
<212> DNA
<213> GLUT12

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<222> (106)..(1959)
<223>

<400> 55

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ggggcggttt ggtggcgggg aagcggctgt aacttctacg tgacc atg gta cct gtt    117
                                                 Met Val Pro Val
                                                 1

gaa aac acc gag ggc ccc agt ctg ctg aac cag aag ggg aca gcc gtg      165
Glu Asn Thr Glu Gly Pro Ser Leu Leu Asn Gln Lys Gly Thr Ala Val
5                   10                  15                  20

gag acg gag ggc agc ggc agc cgg cat cct ccc tgg gcg aga ggc tgc      213
Glu Thr Glu Gly Ser Gly Ser Arg His Pro Pro Trp Ala Arg Gly Cys
                25                  30                  35

ggc atg ttt acc ttc ctg tca tct gtc act gct gct gtc agt ggc ctc      261
Gly Met Phe Thr Phe Leu Ser Ser Val Thr Ala Ala Val Ser Gly Leu
            40                  45                  50

ctg gtg ggt tat gaa ctt ggg atc atc tct ggg gct ctt ctt cag atc      309
Leu Val Gly Tyr Glu Leu Gly Ile Ile Ser Gly Ala Leu Leu Gln Ile
        55                  60                  65

aaa acc tta tta gcc ctg agc tgc cat gag cag gaa atg gtt gtg agc      357
Lys Thr Leu Leu Ala Leu Ser Cys His Glu Gln Glu Met Val Val Ser
    70                  75                  80

tcc ctc gtc att gga gcc ctc ctt gcc tca ctc acc gga ggg gtc ctg      405
Ser Leu Val Ile Gly Ala Leu Leu Ala Ser Leu Thr Gly Gly Val Leu
85                  90                  95                  100

ata gac aga tat gga aga agg aca gca atc atc ttg tca tcc tgc ctg      453
Ile Asp Arg Tyr Gly Arg Arg Thr Ala Ile Ile Leu Ser Ser Cys Leu
                105                 110                 115

ctt gga ctc gga agc tta gtc ttg atc ctc agt tta tcc tac acg gtt      501
```

```
Leu Gly Leu Gly Ser Leu Val Leu Ile Leu Ser Leu Ser Tyr Thr Val
            120                 125                 130

ctt ata gtg gga cgc att gcc ata ggg gtc tcc atc tcc ctc tct tcc      549
Leu Ile Val Gly Arg Ile Ala Ile Gly Val Ser Ile Ser Leu Ser Ser
        135                 140                 145

att gcc act tgt gtt tac atc gca gag att gct cct caa cac aga aga      597
Ile Ala Thr Cys Val Tyr Ile Ala Glu Ile Ala Pro Gln His Arg Arg
    150                 155                 160

ggc ctt ctt gtg tca ctg aat gag ctg atg att gtc atc ggc att ctt      645
Gly Leu Leu Val Ser Leu Asn Glu Leu Met Ile Val Ile Gly Ile Leu
165                 170                 175                 180

tct gcc tat att tca aat tac gca ttt gcc aat gtt ttc cat ggc tgg      693
Ser Ala Tyr Ile Ser Asn Tyr Ala Phe Ala Asn Val Phe His Gly Trp
                185                 190                 195

aag tac atg ttt ggt ctt gtg att ccc ttg gga gtt ttg caa gca att      741
Lys Tyr Met Phe Gly Leu Val Ile Pro Leu Gly Val Leu Gln Ala Ile
            200                 205                 210

gca atg tat ttt ctt cct cca agc cct cgg ttt ctg gtg atg aaa gga      789
Ala Met Tyr Phe Leu Pro Pro Ser Pro Arg Phe Leu Val Met Lys Gly
        215                 220                 225

caa gag gga gct gct agc aag gtt ctt gga agg tta aga gca ctc tca      837
Gln Glu Gly Ala Ala Ser Lys Val Leu Gly Arg Leu Arg Ala Leu Ser
    230                 235                 240

gat aca act gag gaa ctc act gtg atc aaa tcc tcc ctg aaa gat gaa      885
Asp Thr Thr Glu Glu Leu Thr Val Ile Lys Ser Ser Leu Lys Asp Glu
245                 250                 255                 260

tat cag tac agt ttt tgg gat ctg ttt cgt tca aaa gac aac atg cgg      933
Tyr Gln Tyr Ser Phe Trp Asp Leu Phe Arg Ser Lys Asp Asn Met Arg
                265                 270                 275

acc cga ata atg ata gga cta aca cta gta ttt ttt gta caa atc act      981
Thr Arg Ile Met Ile Gly Leu Thr Leu Val Phe Phe Val Gln Ile Thr
            280                 285                 290

ggc caa cca aac ata ttg ttc tat gca tca act gtt ttg aag tca gtt     1029
Gly Gln Pro Asn Ile Leu Phe Tyr Ala Ser Thr Val Leu Lys Ser Val
        295                 300                 305

gga ttt caa agc aat gag gca gct agc ctc gcc tcc act ggg gtt gga     1077
Gly Phe Gln Ser Asn Glu Ala Ala Ser Leu Ala Ser Thr Gly Val Gly
    310                 315                 320

gtc gtc aag gtc att agc acc atc cct gcc act ctt ctt gta gac cat     1125
Val Val Lys Val Ile Ser Thr Ile Pro Ala Thr Leu Leu Val Asp His
325                 330                 335                 340

gtt ggc agc aaa aca ttc ctc tgc att ggc tcc tct gtg atg gca gct     1173
Val Gly Ser Lys Thr Phe Leu Cys Ile Gly Ser Ser Val Met Ala Ala
                345                 350                 355

tcg ttg gtg acc atg ggc atc gta aat ctc aac atc cac atg aac ttc     1221
Ser Leu Val Thr Met Gly Ile Val Asn Leu Asn Ile His Met Asn Phe
            360                 365                 370
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acc cat atc tgc aga agc cac aat tct atc aac cag tcc ttg gat gag     1269
Thr His Ile Cys Arg Ser His Asn Ser Ile Asn Gln Ser Leu Asp Glu
        375                 380                 385

tct gtg att tat gga cca gga aac ctg tca acc aac aac aat act ctc     1317
Ser Val Ile Tyr Gly Pro Gly Asn Leu Ser Thr Asn Asn Asn Thr Leu
    390                 395                 400

aga gac cac ttc aaa ggg att tct tcc cat agc aga agc tca ctc atg     1365
Arg Asp His Phe Lys Gly Ile Ser Ser His Ser Arg Ser Ser Leu Met
405                 410                 415                 420

ccc ctg aga aat gat gtg gat aag aga ggg gag acg acc tca gca tcc     1413
Pro Leu Arg Asn Asp Val Asp Lys Arg Gly Glu Thr Thr Ser Ala Ser
                425                 430                 435

ttg cta aat gct gga tta agc cac act gaa tac cag ata gtc aca gac     1461
Leu Leu Asn Ala Gly Leu Ser His Thr Glu Tyr Gln Ile Val Thr Asp
            440                 445                 450

cct ggg gac gtc cca gct ttt ttg aaa tgg ctg tcc tta gcc agc ttg     1509
Pro Gly Asp Val Pro Ala Phe Leu Lys Trp Leu Ser Leu Ala Ser Leu
        455                 460                 465

ctt gtt tat gtt gct gct ttt tca att ggt cta gga cca atg ccc tgg     1557
Leu Val Tyr Val Ala Ala Phe Ser Ile Gly Leu Gly Pro Met Pro Trp
    470                 475                 480

ctg gtg ctc agc gag atc ttt cct ggt ggg atc aga gga cga gcc atg     1605
Leu Val Leu Ser Glu Ile Phe Pro Gly Gly Ile Arg Gly Arg Ala Met
485                 490                 495                 500

gct tta act tct agc atg aac tgg ggc atc aat ctc ctc atc tcg ctg     1653
Ala Leu Thr Ser Ser Met Asn Trp Gly Ile Asn Leu Leu Ile Ser Leu
                505                 510                 515

aca ttt ttg act gta act gat ctt att ggc ctg cca tgg gtg tgc ttt     1701
Thr Phe Leu Thr Val Thr Asp Leu Ile Gly Leu Pro Trp Val Cys Phe
            520                 525                 530

ata tat aca atc atg agt cta gca tcc ctg ctt ttt gtt gtt atg ttt     1749
Ile Tyr Thr Ile Met Ser Leu Ala Ser Leu Leu Phe Val Val Met Phe
        535                 540                 545

ata cct gag aca aag gga tgc tct ttg gaa caa ata tca atg gag cta     1797
Ile Pro Glu Thr Lys Gly Cys Ser Leu Glu Gln Ile Ser Met Glu Leu
    550                 555                 560

gca aaa gtg aac tat gtg aaa aac aac att tgt ttt atg agt cat cac     1845
Ala Lys Val Asn Tyr Val Lys Asn Asn Ile Cys Phe Met Ser His His
565                 570                 575                 580

caa gaa gaa tta gtg cca aaa cag cct caa aaa aga aaa ccc cag gag     1893
Gln Glu Glu Leu Val Pro Lys Gln Pro Gln Lys Arg Lys Pro Gln Glu
                585                 590                 595

cag ctc ttg gag tgt aac aag ctg tgt ggt agg ggc caa tcc agg cag     1941
Gln Leu Leu Glu Cys Asn Lys Leu Cys Gly Arg Gly Gln Ser Arg Gln
            600                 605                 610

ctt tct cca gag acc taa tggcctcaac accttctgaa cgtggatagt           1989
Leu Ser Pro Glu Thr
        615
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gccagaacac ttaggagggt gtctttggac caatgcatag ttgcgactcc tgtgctctct 2049

tttcagtgtc atggaactgg ttttgaagag acactctgaa atgataaaga cagcctttaa 2109

tccccctcct ccccagaagg aacctcaaaa ggtagatgag gtacaaggtc ctaagtgatc 2169

tctttttctg agcaggatat caggcaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 2223

<210> 56
<211> 617
<212> PRT
<213> GLUT12

<400> 56

Met Val Pro Val Glu Asn Thr Glu Gly Pro Ser Leu Leu Asn Gln Lys
1 5 10 15

Gly Thr Ala Val Glu Thr Glu Gly Ser Gly Ser Arg His Pro Pro Trp
20 25 30

Ala Arg Gly Cys Gly Met Phe Thr Phe Leu Ser Ser Val Thr Ala Ala
35 40 45

Val Ser Gly Leu Leu Val Gly Tyr Glu Leu Gly Ile Ile Ser Gly Ala
50 55 60

Leu Leu Gln Ile Lys Thr Leu Leu Ala Leu Ser Cys His Glu Gln Glu
65 70 75 80

Met Val Val Ser Ser Leu Val Ile Gly Ala Leu Leu Ala Ser Leu Thr
85 90 95

Gly Gly Val Leu Ile Asp Arg Tyr Gly Arg Arg Thr Ala Ile Ile Leu
100 105 110

Ser Ser Cys Leu Leu Gly Leu Gly Ser Leu Val Leu Ile Leu Ser Leu
115 120 125

Ser Tyr Thr Val Leu Ile Val Gly Arg Ile Ala Ile Gly Val Ser Ile
130 135 140

Ser Leu Ser Ser Ile Ala Thr Cys Val Tyr Ile Ala Glu Ile Ala Pro
145 150 155 160

Gln His Arg Arg Gly Leu Leu Val Ser Leu Asn Glu Leu Met Ile Val
165 170 175

Ile Gly Ile Leu Ser Ala Tyr Ile Ser Asn Tyr Ala Phe Ala Asn Val
180 185 190

Phe His Gly Trp Lys Tyr Met Phe Gly Leu Val Ile Pro Leu Gly Val
195 200 205

Leu Gln Ala Ile Ala Met Tyr Phe Leu Pro Pro Ser Pro Arg Phe Leu
210 215 220

Val Met Lys Gly Gln Glu Gly Ala Ala Ser Lys Val Leu Gly Arg Leu
225 230 235 240

Arg Ala Leu Ser Asp Thr Thr Glu Glu Leu Thr Val Ile Lys Ser Ser
245 250 255

Leu Lys Asp Glu Tyr Gln Tyr Ser Phe Trp Asp Leu Phe Arg Ser Lys
260 265 270

Asp Asn Met Arg Thr Arg Ile Met Ile Gly Leu Thr Leu Val Phe Phe
275 280 285

Val Gln Ile Thr Gly Gln Pro Asn Ile Leu Phe Tyr Ala Ser Thr Val
290 295 300

Leu Lys Ser Val Gly Phe Gln Ser Asn Glu Ala Ala Ser Leu Ala Ser
305 310 315 320

Thr Gly Val Gly Val Val Lys Val Ile Ser Thr Ile Pro Ala Thr Leu
325 330 335

Leu Val Asp His Val Gly Ser Lys Thr Phe Leu Cys Ile Gly Ser Ser
340 345 350

Val Met Ala Ala Ser Leu Val Thr Met Gly Ile Val Asn Leu Asn Ile
355 360 365

His Met Asn Phe Thr His Ile Cys Arg Ser His Asn Ser Ile Asn Gln
370 375 380

Ser Leu Asp Glu Ser Val Ile Tyr Gly Pro Gly Asn Leu Ser Thr Asn
385 390 395 400

Asn Asn Thr Leu Arg Asp His Phe Lys Gly Ile Ser Ser His Ser Arg
405 410 415

Ser Ser Leu Met Pro Leu Arg Asn Asp Val Asp Lys Arg Gly Glu Thr
420 425 430

Thr Ser Ala Ser Leu Leu Asn Ala Gly Leu Ser His Thr Glu Tyr Gln

435 440 445

Ile Val Thr Asp Pro Gly Asp Val Pro Ala Phe Leu Lys Trp Leu Ser
450 455 460

Leu Ala Ser Leu Leu Val Tyr Val Ala Ala Phe Ser Ile Gly Leu Gly
465 470 475 480

Pro Met Pro Trp Leu Val Leu Ser Glu Ile Phe Pro Gly Gly Ile Arg
485 490 495

Gly Arg Ala Met Ala Leu Thr Ser Ser Met Asn Trp Gly Ile Asn Leu
500 505 510

Leu Ile Ser Leu Thr Phe Leu Thr Val Thr Asp Leu Ile Gly Leu Pro
515 520 525

Trp Val Cys Phe Ile Tyr Thr Ile Met Ser Leu Ala Ser Leu Leu Phe
530 535 540

Val Val Met Phe Ile Pro Glu Thr Lys Gly Cys Ser Leu Glu Gln Ile
545 550 555 560

Ser Met Glu Leu Ala Lys Val Asn Tyr Val Lys Asn Asn Ile Cys Phe
565 570 575

Met Ser His His Gln Glu Glu Leu Val Pro Lys Gln Pro Gln Lys Arg
580 585 590

Lys Pro Gln Glu Gln Leu Leu Glu Cys Asn Lys Leu Cys Gly Arg Gly
595 600 605

Gln Ser Arg Gln Leu Ser Pro Glu Thr
610 615

<210> 57
<211> 3261
<212> DNA
<213> GLUT13 (HMIT)

<220>
<221> CDS
<222> (109)..(1998)
<223>

<400> 57
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aaggcaagcg agaatgtgga gtacacgctg cggagcctga gcagcctg atg ggc gag 117
Met Gly Glu
1

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cgg cgc agg aag cag ccg gag ccg gac gcg gcg agc gcg gcc ggg gag      165
Arg Arg Arg Lys Gln Pro Glu Pro Asp Ala Ala Ser Ala Ala Gly Glu
    5                   10                  15

tgc agc ctc ctg gct gcc gcc gaa tcg agc acc agc ctg cag agc gcg      213
Cys Ser Leu Leu Ala Ala Ala Glu Ser Ser Thr Ser Leu Gln Ser Ala
20                  25                  30                  35

ggc gcg ggc ggc ggc ggc gtc ggg gac ctg gag cgc gcg gcg cgg cgg      261
Gly Ala Gly Gly Gly Gly Val Gly Asp Leu Glu Arg Ala Ala Arg Arg
                40                  45                  50

cag ttc cag cag gac gag acc ccc gcc ttc gtg tac gtg gtg gcc gtc      309
Gln Phe Gln Gln Asp Glu Thr Pro Ala Phe Val Tyr Val Val Ala Val
            55                  60                  65

ttc tcc gcg ctg ggc ggc ttc ctg ttt ggc tat gac acc ggg gtg gtg      357
Phe Ser Ala Leu Gly Gly Phe Leu Phe Gly Tyr Asp Thr Gly Val Val
        70                  75                  80

tca ggg gcc atg ctg ctg ctc aag cgg cag ctc agt ctg gac gcg ctg      405
Ser Gly Ala Met Leu Leu Leu Lys Arg Gln Leu Ser Leu Asp Ala Leu
    85                  90                  95

tgg cag gag ctg ctg gtg tcc agc acg gtg ggg gcg gct gcc gtc tcg      453
Trp Gln Glu Leu Leu Val Ser Ser Thr Val Gly Ala Ala Ala Val Ser
100                 105                 110                 115

gcg ctg gcc gga ggc gcc ctc aac ggc gtc ttc ggc cgc cgc gct gcc      501
Ala Leu Ala Gly Gly Ala Leu Asn Gly Val Phe Gly Arg Arg Ala Ala
                120                 125                 130

atc ctc ctg gcc agt gcc ctc ttc acc gcc ggc tcc gcg gtg ctg gct      549
Ile Leu Leu Ala Ser Ala Leu Phe Thr Ala Gly Ser Ala Val Leu Ala
            135                 140                 145

gcg gcc aac aac aag gag aca ctg ctc gcc ggc cgc ctg gtc gtg gga      597
Ala Ala Asn Asn Lys Glu Thr Leu Leu Ala Gly Arg Leu Val Val Gly
        150                 155                 160

ctc ggc atc ggc att gct tct atg aca gtg cca gtg tac att gcg gag      645
Leu Gly Ile Gly Ile Ala Ser Met Thr Val Pro Val Tyr Ile Ala Glu
    165                 170                 175

gtc tca cca ccc aat tta aga ggc cga tta gtc acc att aat acc ctc      693
Val Ser Pro Pro Asn Leu Arg Gly Arg Leu Val Thr Ile Asn Thr Leu
180                 185                 190                 195

ttc atc aca gga ggg cag ttc ttt gca agt gtt gtt gat gga gcc ttc      741
Phe Ile Thr Gly Gly Gln Phe Phe Ala Ser Val Val Asp Gly Ala Phe
                200                 205                 210

agt tat ctc cag aag gat gga tgg agg tac atg ttg gga ctt gca rca      789
Ser Tyr Leu Gln Lys Asp Gly Trp Arg Tyr Met Leu Gly Leu Ala Xaa
            215                 220                 225

gtt ccg gcg gtt ata cag ttt ttt ggc ttt ctc ttt ttg cct gaa agc      837
Val Pro Ala Val Ile Gln Phe Phe Gly Phe Leu Phe Leu Pro Glu Ser
        230                 235                 240

cct cga tgg ctt att cag aaa gga cag act cag aag gcc cgt aga att      885
Pro Arg Trp Leu Ile Gln Lys Gly Gln Thr Gln Lys Ala Arg Arg Ile
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    245                 250                 255

tta tct cag atg cgt ggt aac cag acc att gat gag gaa tat gat agc      933
Leu Ser Gln Met Arg Gly Asn Gln Thr Ile Asp Glu Glu Tyr Asp Ser
260                 265                 270                 275

atc aaa aac aac att gaa gag gag gaa aaa gag gtt ggc tca gct gga      981
Ile Lys Asn Asn Ile Glu Glu Glu Glu Lys Glu Val Gly Ser Ala Gly
                280                 285                 290

cct gtg atc tgc aga atg ctg agt tat ccc cca act cgc cga gct tta     1029
Pro Val Ile Cys Arg Met Leu Ser Tyr Pro Pro Thr Arg Arg Ala Leu
            295                 300                 305

att gtg ggt tgt ggc cta caa atg ttc cag cag ctc tca ggc att aac     1077
Ile Val Gly Cys Gly Leu Gln Met Phe Gln Gln Leu Ser Gly Ile Asn
        310                 315                 320

acc atc atg tac tac agt gca acc att ctg cag atg tct ggt gtt gaa     1125
Thr Ile Met Tyr Tyr Ser Ala Thr Ile Leu Gln Met Ser Gly Val Glu
    325                 330                 335

gat gat aga ctt gca ata tgg ctg gct tca gtt aca gcc ttc aca aat     1173
Asp Asp Arg Leu Ala Ile Trp Leu Ala Ser Val Thr Ala Phe Thr Asn
340                 345                 350                 355

ttc att ttc aca ctt gtg gga gtc tgg ctt gtt gag aag gtg ggc cgc     1221
Phe Ile Phe Thr Leu Val Gly Val Trp Leu Val Glu Lys Val Gly Arg
                360                 365                 370

aga aag ctt acc ttt ggt agt tta gca ggt acc acc gta gca ctc att     1269
Arg Lys Leu Thr Phe Gly Ser Leu Ala Gly Thr Thr Val Ala Leu Ile
            375                 380                 385

att ctt gcc ttg gga ttt gtg cta tca gcc caa gtt tcc cca cgc atc     1317
Ile Leu Ala Leu Gly Phe Val Leu Ser Ala Gln Val Ser Pro Arg Ile
        390                 395                 400

act ttt aag cca ata gct ccg tca ggt cag aac gcc act tgc aca aga     1365
Thr Phe Lys Pro Ile Ala Pro Ser Gly Gln Asn Ala Thr Cys Thr Arg
    405                 410                 415

tac agt tac tgt aat gaa tgt atg ttg gat cca gac tgc ggt ttc tgc     1413
Tyr Ser Tyr Cys Asn Glu Cys Met Leu Asp Pro Asp Cys Gly Phe Cys
420                 425                 430                 435

twc aag atg aac aaa tca act gtc att gac tcc tcc tgt gtt cca gtt     1461
Xaa Lys Met Asn Lys Ser Thr Val Ile Asp Ser Ser Cys Val Pro Val
                440                 445                 450

aat aaa gca tct aca aat gag gca gcc tgg ggc agg tgt gaa aat gaa     1509
Asn Lys Ala Ser Thr Asn Glu Ala Ala Trp Gly Arg Cys Glu Asn Glu
            455                 460                 465

acc aag ttc aaa aca gaa gat ata ttt tgg gct tac aat ttc tgc cct     1557
Thr Lys Phe Lys Thr Glu Asp Ile Phe Trp Ala Tyr Asn Phe Cys Pro
        470                 475                 480

act cca tac tcc tgg act gca ctt ctg ggc ctt att tta tat ctt gtc     1605
Thr Pro Tyr Ser Trp Thr Ala Leu Leu Gly Leu Ile Leu Tyr Leu Val
    485                 490                 495

ttc ttt gca cct gga atg gga cca atg cct tgg act gtg aat tct gaa     1653
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Phe Phe Ala Pro Gly Met Gly Pro Met Pro Trp Thr Val Asn Ser Glu
500                 505                 510                 515

ata tat ccc ctt tgg gca aga agt aca gga aat gca tgt tca tct gga     1701
Ile Tyr Pro Leu Trp Ala Arg Ser Thr Gly Asn Ala Cys Ser Ser Gly
                520                 525                 530

ata aac tgg att ttc aat gtc ctg gtt tca cta aca ttt tta cac aca     1749
Ile Asn Trp Ile Phe Asn Val Leu Val Ser Leu Thr Phe Leu His Thr
            535                 540                 545

gca gag tat ctt aca tac tat gga gct ttc ttc ctc tat gct gga ttt     1797
Ala Glu Tyr Leu Thr Tyr Tyr Gly Ala Phe Phe Leu Tyr Ala Gly Phe
        550                 555                 560

gct gct gtg gga ctc ctt ttc atc tat ggc tgt ctt cct gag acc aaa     1845
Ala Ala Val Gly Leu Leu Phe Ile Tyr Gly Cys Leu Pro Glu Thr Lys
    565                 570                 575

ggc aaa aaa tta gag gaa att gaa tca ctc ttt gac aac agg cta tgt     1893
Gly Lys Lys Leu Glu Glu Ile Glu Ser Leu Phe Asp Asn Arg Leu Cys
580                 585                 590                 595

aca tgt ggc act tca gat tct gat gaa ggg aga tat att gaa tat att     1941
Thr Cys Gly Thr Ser Asp Ser Asp Glu Gly Arg Tyr Ile Glu Tyr Ile
                600                 605                 610

cgg gta aag gga agt aac tat cat ctt tct gac aat gat gct tct gat     1989
Arg Val Lys Gly Ser Asn Tyr His Leu Ser Asp Asn Asp Ala Ser Asp
            615                 620                 625

gtg gaa taa ttttcagctg ctcatatatt tagttattta aacaaactgg             2038
Val Glu

ggggagaaga acagcaattg gtgacttcac tgccctgctt ctaatctggt tctttccaca   2098

gcctagtttt gattgacttc atattctaga atacttgatt aggaggaaga tacaaccatg   2158

atgacttttt ttttccacaa ggaacaatat tttaaaaaat atttacagag attttaatct   2218

aataattctt aagcaaatgt gtgtaatgcc ttcctgaaat agtctaaaat gaatattgta   2278

cccagtgact tcagtggtat ccttttttcc taagaccatt tataattatt agtggcaaca   2338

gagtcagtgc taatctagcc aaattacata tgtataatat atttataaag gattctggga   2398

gatggtccaa gggtgttctg tgtcaaaaga tggcctattg gccctcagtt ttcctacaga   2458

gtagtggctt atctctgatc agctgttaca aactaaattc catgtaagct ttcatcaaca   2518

aattccaaag tgcctcctac aagggcacag ctgtccgtat ctcctttgga ttccatattt   2578

ttgtttctct ccaattcaga tattgggagt tcttcagata ctgactctgc acactatctt   2638

ttgaacatta tgaaaatata atctgtcggg ttgttttcat acttccttt tttgcatcag   2698

tgataaggca tacatttgcg cagatgagct aggaagtttt gagaagtacc ttttatgttc   2758

agaaagtaac agattaaagg tttttatggg ttccaaattg gttagtcact taggtatgca   2818

attataaatg caaaatagtg ccagatcttt aactgtatat cagggtcagt ttttgttcat   2878
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gcccaaatca ctacaccttc ctcattggcc ttggagtcct actattttgt gccttcattt 2938

gtgttacata catagctgca agcaaaccat ttttcccctt tcttttattc aaacaataat 2998

ttttgaaaca aaaaagagga aggaaatcag tggcagaaat aatcctgctg ttattggtgt 3058

ttgtttaata aaaataatgg gacttttttc ttaacttttt attagctctt cctaagggaa 3118

atgtcacata ttattattta attgtacttg tctttttttа ctttaagagc ataaactcgt 3178

ttttattttg cacacttttc tcattttcct gagaatttac cagaaaaaaa aagatacata 3238

gatttgtctc tgtgtttttc tta 3261

<210> 58
<211> 629
<212> PRT
<213> GLUT13 (HMIT)

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<221> misc_feature
<222> (227)..(227)
<223> The 'Xaa' at location 227 stands for Ala, or Thr.

<220>
<221> misc_feature
<222> (436)..(436)
<223> The 'Xaa' at location 436 stands for Tyr, or Phe.

<400> 58

Met Gly Glu Arg Arg Arg Lys Gln Pro Glu Pro Asp Ala Ala Ser Ala
1 5 10 15

Ala Gly Glu Cys Ser Leu Leu Ala Ala Ala Glu Ser Ser Thr Ser Leu
20 25 30

Gln Ser Ala Gly Ala Gly Gly Gly Gly Val Gly Asp Leu Glu Arg Ala
35 40 45

Ala Arg Arg Gln Phe Gln Gln Asp Glu Thr Pro Ala Phe Val Tyr Val
50 55 60

Val Ala Val Phe Ser Ala Leu Gly Gly Phe Leu Phe Gly Tyr Asp Thr
65 70 75 80

Gly Val Val Ser Gly Ala Met Leu Leu Leu Lys Arg Gln Leu Ser Leu
85 90 95

Asp Ala Leu Trp Gln Glu Leu Leu Val Ser Ser Thr Val Gly Ala Ala
100 105 110

Ala Val Ser Ala Leu Ala Gly Gly Ala Leu Asn Gly Val Phe Gly Arg
115 120 125

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Arg Ala Ala Ile Leu Leu Ala Ser Ala Leu Phe Thr Ala Gly Ser Ala
    130                 135                 140

Val Leu Ala Ala Ala Asn Asn Lys Glu Thr Leu Leu Ala Gly Arg Leu
145                 150                 155                 160

Val Val Gly Leu Gly Ile Gly Ile Ala Ser Met Thr Val Pro Val Tyr
                165                 170                 175

Ile Ala Glu Val Ser Pro Pro Asn Leu Arg Gly Arg Leu Val Thr Ile
            180                 185                 190

Asn Thr Leu Phe Ile Thr Gly Gly Gln Phe Phe Ala Ser Val Val Asp
        195                 200                 205

Gly Ala Phe Ser Tyr Leu Gln Lys Asp Gly Trp Arg Tyr Met Leu Gly
    210                 215                 220

Leu Ala Xaa Val Pro Ala Val Ile Gln Phe Phe Gly Phe Leu Phe Leu
225                 230                 235                 240

Pro Glu Ser Pro Arg Trp Leu Ile Gln Lys Gly Gln Thr Gln Lys Ala
                245                 250                 255

Arg Arg Ile Leu Ser Gln Met Arg Gly Asn Gln Thr Ile Asp Glu Glu
            260                 265                 270

Tyr Asp Ser Ile Lys Asn Asn Ile Glu Glu Glu Glu Lys Glu Val Gly
        275                 280                 285

Ser Ala Gly Pro Val Ile Cys Arg Met Leu Ser Tyr Pro Pro Thr Arg
    290                 295                 300

Arg Ala Leu Ile Val Gly Cys Gly Leu Gln Met Phe Gln Gln Leu Ser
305                 310                 315                 320

Gly Ile Asn Thr Ile Met Tyr Tyr Ser Ala Thr Ile Leu Gln Met Ser
                325                 330                 335

Gly Val Glu Asp Asp Arg Leu Ala Ile Trp Leu Ala Ser Val Thr Ala
            340                 345                 350

Phe Thr Asn Phe Ile Phe Thr Leu Val Gly Val Trp Leu Val Glu Lys
        355                 360                 365

Val Gly Arg Arg Lys Leu Thr Phe Gly Ser Leu Ala Gly Thr Thr Val
    370                 375                 380
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Ala Leu Ile Ile Leu Ala Leu Gly Phe Val Leu Ser Ala Gln Val Ser
385                 390                 395                 400

Pro Arg Ile Thr Phe Lys Pro Ile Ala Pro Ser Gly Gln Asn Ala Thr
                405                 410                 415

Cys Thr Arg Tyr Ser Tyr Cys Asn Glu Cys Met Leu Asp Pro Asp Cys
            420                 425                 430

Gly Phe Cys Xaa Lys Met Asn Lys Ser Thr Val Ile Asp Ser Ser Cys
        435                 440                 445

Val Pro Val Asn Lys Ala Ser Thr Asn Glu Ala Ala Trp Gly Arg Cys
    450                 455                 460

Glu Asn Glu Thr Lys Phe Lys Thr Glu Asp Ile Phe Trp Ala Tyr Asn
465                 470                 475                 480

Phe Cys Pro Thr Pro Tyr Ser Trp Thr Ala Leu Leu Gly Leu Ile Leu
                485                 490                 495

Tyr Leu Val Phe Phe Ala Pro Gly Met Gly Pro Met Pro Trp Thr Val
            500                 505                 510

Asn Ser Glu Ile Tyr Pro Leu Trp Ala Arg Ser Thr Gly Asn Ala Cys
        515                 520                 525

Ser Ser Gly Ile Asn Trp Ile Phe Asn Val Leu Val Ser Leu Thr Phe
    530                 535                 540

Leu His Thr Ala Glu Tyr Leu Thr Tyr Tyr Gly Ala Phe Phe Leu Tyr
545                 550                 555                 560

Ala Gly Phe Ala Ala Val Gly Leu Leu Phe Ile Tyr Gly Cys Leu Pro
                565                 570                 575

Glu Thr Lys Gly Lys Lys Leu Glu Glu Ile Glu Ser Leu Phe Asp Asn
            580                 585                 590

Arg Leu Cys Thr Cys Gly Thr Ser Asp Ser Asp Glu Gly Arg Tyr Ile
        595                 600                 605

Glu Tyr Ile Arg Val Lys Gly Ser Asn Tyr His Leu Ser Asp Asn Asp
    610                 615                 620

Ala Ser Asp Val Glu
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625

<210> 59
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<221> CDS
<222> (110)..(1603)
<223>

<400> 59
agcggggctg agcgaagccg cggggcccaa ccgcagtcgc ggggtctggg aggaagcagt 60

accttgaaga gaaattggag agggagtcaa ttcctaggat agcagagag atg gac aac 118
Met Asp Asn
1

aga cag aat gtc acc cca gct ctg atc ttt gcc atc aca gtt gct aca 166
Arg Gln Asn Val Thr Pro Ala Leu Ile Phe Ala Ile Thr Val Ala Thr
5 10 15

atc ggc tct ttc cag ttt ggc tac aac act ggg gtc atc aat gct cct 214
Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile Asn Ala Pro
20 25 30 35

gag acg atc ata aag gaa ttt atc aat aaa act ttg acg gac aag gca 262
Glu Thr Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr Asp Lys Ala
40 45 50

aat gcc cct ccc tct gag gtg ctg ctc acg aat ctc tgg tcc ttg tct 310
Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Asn Leu Trp Ser Leu Ser
55 60 65

gtg gcc ata ttt tcc gtc ggg ggt atg atc ggc tcc ttt tcc gtc gga 358
Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe Ser Val Gly
70 75 80

ctc ttt gtt aac cgc ttt ggc agg cgc aat tca atg ctg att gtc aac 406
Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu Ile Val Asn
85 90 95

ctg ttg gct gcc act ggt ggc tgc ctt atg gga ctg tgt aaa ata gct 454
Leu Leu Ala Ala Thr Gly Gly Cys Leu Met Gly Leu Cys Lys Ile Ala
100 105 110 115

gag tca gtt gaa atg ctg atc ctg ggc cgc ttg gtt att ggc ctc ttc 502
Glu Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile Gly Leu Phe
120 125 130

tgc gga ctc tgc aca ggt ttt gtg ccc atg tac att gga gag atc tcg 550
Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly Glu Ile Ser
135 140 145

cct act gcc ctg agg ggt gcc ttt ggc act ctc aac cag ctg ggc ata 598
Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln Leu Gly Ile
150 155 160

gtt att gga att ctg gtg gcc cag atc ttt ggt ctg gaa ctc atc ctt 646
Val Ile Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu Leu Ile Leu
165 170 175

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ggg tct gaa gag cta tgg ccg gtg cta tta ggc ttt acc atc ctt cca      694
Gly Ser Glu Glu Leu Trp Pro Val Leu Leu Gly Phe Thr Ile Leu Pro
180                 185                 190                 195

gct atc ctg caa agt gca gcc ctt cca tgt tgc cct gaa agt ccc aga      742
Ala Ile Leu Gln Ser Ala Ala Leu Pro Cys Cys Pro Glu Ser Pro Arg
                200                 205                 210

ttt ttg ctc att aac aga aaa aaa gag gag aat gct acg cgg atc ctc      790
Phe Leu Leu Ile Asn Arg Lys Lys Glu Glu Asn Ala Thr Arg Ile Leu
            215                 220                 225

cag cgg ttg tgg ggc acc cag gat gta tcc caa gac atc cag gag atg      838
Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile Gln Glu Met
        230                 235                 240

aaa gat gag agt gca agg atg tca caa gaa aag caa gtc acc gtg ctg      886
Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val Thr Val Leu
    245                 250                 255

gag ctc ttt aga gtg tcc agc tac cga cag ccc atc atc att tcc att      934
Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile Ile Ser Ile
260                 265                 270                 275

gtg ctc cag ctc tct cag cag ctc tct ggg atc aat gct gtg ttc tat      982
Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val Phe Tyr
                280                 285                 290

tac tca aca gga atc ttc aag gat gca ggt gtt caa cag ccc atc tat     1030
Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Gln Pro Ile Tyr
            295                 300                 305

gcc acc atc agc gcg ggt gtg gtt aat act atc ttc act tta ctt tct     1078
Ala Thr Ile Ser Ala Gly Val Val Asn Thr Ile Phe Thr Leu Leu Ser
        310                 315                 320

cta ttt ctg gtg gaa agg gca gga aga agg act ctg cat atg ata ggc     1126
Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Met Ile Gly
    325                 330                 335

ctt gga ggg atg gct ttt tgt tcc acg ctc atg act gtt tct ttg tta     1174
Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val Ser Leu Leu
340                 345                 350                 355

tta aag aat cac tat aat ggg atg agc ttt gtc tgt att ggg gct atc     1222
Leu Lys Asn His Tyr Asn Gly Met Ser Phe Val Cys Ile Gly Ala Ile
                360                 365                 370

ttg gtc ttt gtg gcc tgt ttt gaa att gga cca ggc ccc att ccc tgg     1270
Leu Val Phe Val Ala Cys Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp
            375                 380                 385

ttt att gtg gcc gaa ctc ttc agc cag ggc ccc cgc cca gct gcg atg     1318
Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala Met
        390                 395                 400

gca gtg gcc ggc tgc tcc aac tgg acc tcc aac ttc cta gtc gga ttg     1366
Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu Val Gly Leu
    405                 410                 415

ctc ttc ccc tct gct gct tac tat tta gga gcc tac gtt ttt att atc     1414
Leu Phe Pro Ser Ala Ala Tyr Tyr Leu Gly Ala Tyr Val Phe Ile Ile
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420                 425                 430                 435

ttc acc ggc ttc ctc att acc ttc ttg gcc ttt acc ttc ttc aaa gtc     1462
Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe Phe Lys Val
                440                 445                 450

cct gag acc cgt ggc agg act ttt gag gat atc aca cgg gcc ttt gaa     1510
Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg Ala Phe Glu
            455                 460                 465

ggg cag gca cac ggt gca gat aga tct ggg aag gac ggc gtc atg ggg     1558
Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly Val Met Gly
        470                 475                 480

atg aac agc atc gag cct gct aag gag acc acc acc aat gtc taa         1603
Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn Val
    485                 490                 495

gtcatgcctc cttccacctc cctcccggca tgggaaagcc acctctccct caacaaggga   1663

gagactttat caggatgaac ccaggactgc ttctgaatgc tgctacttga tttctttctc   1723

atcccacgca ctccatgagc accccaaggc tgcagtttgt tggatcttca atggcttttt   1783

aaatttttatt tcctggacat cctcttctgc ttaggagaga ccgagtgaac ctaccttcat   1843

ttcaggaggg attggccgct tggcacatga caactttgcc agcttttcct cccttgggtt   1903

ctgatattgc cgcactagag gatataggag aggaaaagta aggtgcagtt gccccaacct   1963

cagacttacc aggaagcaga tacatatgag tgtggaagcc ggagggtgtt tatgtaagag   2023

caccttcctc acttccatac agctctacgc ggcaaattaa cttgagtttt atttatctta   2083

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Val Ala Thr Ile Gly Ser Phe Gln Phe Gly Tyr Asn Thr Gly Val Ile
            20                  25                  30

Asn Ala Pro Glu Thr Ile Ile Lys Glu Phe Ile Asn Lys Thr Leu Thr
        35                  40                  45

Asp Lys Ala Asn Ala Pro Pro Ser Glu Val Leu Leu Thr Asn Leu Trp
    50                  55                  60

Ser Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Gly Ser Phe
65                  70                  75                  80
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Ser Val Gly Leu Phe Val Asn Arg Phe Gly Arg Arg Asn Ser Met Leu
85 90 95

Ile Val Asn Leu Leu Ala Ala Thr Gly Gly Cys Leu Met Gly Leu Cys
100 105 110

Lys Ile Ala Glu Ser Val Glu Met Leu Ile Leu Gly Arg Leu Val Ile
115 120 125

Gly Leu Phe Cys Gly Leu Cys Thr Gly Phe Val Pro Met Tyr Ile Gly
130 135 140

Glu Ile Ser Pro Thr Ala Leu Arg Gly Ala Phe Gly Thr Leu Asn Gln
145 150 155 160

Leu Gly Ile Val Ile Gly Ile Leu Val Ala Gln Ile Phe Gly Leu Glu
165 170 175

Leu Ile Leu Gly Ser Glu Glu Leu Trp Pro Val Leu Leu Gly Phe Thr
180 185 190

Ile Leu Pro Ala Ile Leu Gln Ser Ala Ala Leu Pro Cys Cys Pro Glu
195 200 205

Ser Pro Arg Phe Leu Leu Ile Asn Arg Lys Lys Glu Glu Asn Ala Thr
210 215 220

Arg Ile Leu Gln Arg Leu Trp Gly Thr Gln Asp Val Ser Gln Asp Ile
225 230 235 240

Gln Glu Met Lys Asp Glu Ser Ala Arg Met Ser Gln Glu Lys Gln Val
245 250 255

Thr Val Leu Glu Leu Phe Arg Val Ser Ser Tyr Arg Gln Pro Ile Ile
260 265 270

Ile Ser Ile Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala
275 280 285

Val Phe Tyr Tyr Ser Thr Gly Ile Phe Lys Asp Ala Gly Val Gln Gln
290 295 300

Pro Ile Tyr Ala Thr Ile Ser Ala Gly Val Val Asn Thr Ile Phe Thr
305 310 315 320

Leu Leu Ser Leu Phe Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His
325 330 335

Met Ile Gly Leu Gly Gly Met Ala Phe Cys Ser Thr Leu Met Thr Val
340 345 350

Ser Leu Leu Leu Lys Asn His Tyr Asn Gly Met Ser Phe Val Cys Ile
355 360 365

Gly Ala Ile Leu Val Phe Val Ala Cys Phe Glu Ile Gly Pro Gly Pro
370 375 380

Ile Pro Trp Phe Ile Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro
385 390 395 400

Ala Ala Met Ala Val Ala Gly Cys Ser Asn Trp Thr Ser Asn Phe Leu
405 410 415

Val Gly Leu Leu Phe Pro Ser Ala Ala Tyr Tyr Leu Gly Ala Tyr Val
420 425 430

Phe Ile Ile Phe Thr Gly Phe Leu Ile Thr Phe Leu Ala Phe Thr Phe
435 440 445

Phe Lys Val Pro Glu Thr Arg Gly Arg Thr Phe Glu Asp Ile Thr Arg
450 455 460

Ala Phe Glu Gly Gln Ala His Gly Ala Asp Arg Ser Gly Lys Asp Gly
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Val Met Gly Met Asn Ser Ile Glu Pro Ala Lys Glu Thr Thr Thr Asn
485 490 495

Val

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gcccgagaga cc atg cag agg tcg cct ctg gaa aag gcc agc gtt gtc tcc 171

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                Met Gln Arg Ser Pro Leu Glu Lys Ala Ser Val Val Ser
                1               5                   10

aaa ctt ttt ttc agc tgg acc aga cca att ttg agg aaa gga tac aga      219
Lys Leu Phe Phe Ser Trp Thr Arg Pro Ile Leu Arg Lys Gly Tyr Arg
    15                  20                  25

cag cgc ctg gaa ttg tca gac ata tac caa atc cct tct gtt gat tct      267
Gln Arg Leu Glu Leu Ser Asp Ile Tyr Gln Ile Pro Ser Val Asp Ser
30                  35                  40                  45

gct gac aat cta tct gaa aaa ttg gaa aga gaa tgg gat aga gag ctg      315
Ala Asp Asn Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu
                50                  55                  60

gct tca aag aaa aat cct aaa ctc att aat gcc ctt cgg cga tgt ttt      363
Ala Ser Lys Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe
            65                  70                  75

ttc tgg aga ttt atg ttc tat gga atc ttt tta tat tta ggg gaa gtc      411
Phe Trp Arg Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val
        80                  85                  90

acc aaa gca gta cag cct ctc tta ctg gga aga atc ata gct tcc tat      459
Thr Lys Ala Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr
    95                  100                 105

gac ccg gat aac aag gag gaa cgc tct atc gcg att tat cta ggc ata      507
Asp Pro Asp Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile
110                 115                 120                 125

ggc tta tgc ctt ctc ttt att gtg agg aca ctg ctc cta cac cca gcc      555
Gly Leu Cys Leu Leu Phe Ile Val Arg Thr Leu Leu Leu His Pro Ala
                130                 135                 140

att ttt ggc ctt cat cac att gga atg cag atg aga ata gct atg ttt      603
Ile Phe Gly Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe
            145                 150                 155

agt ttg att tat aag aag act tta aag ctg tca agc cgt gtt cta gat      651
Ser Leu Ile Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp
        160                 165                 170

aaa ata agt att gga caa ctt gtt agt ctc ctt tcc aac aac ctg aac      699
Lys Ile Ser Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn
    175                 180                 185

aaa ttt gat gaa gga ctt gca ttg gca cat ttc gtg tgg atc gct cct      747
Lys Phe Asp Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro
190                 195                 200                 205

ttg caa gtg gca ctc ctc atg ggg cta atc tgg gag ttg tta cag gcg      795
Leu Gln Val Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala
                210                 215                 220

tct gcc ttc tgt gga ctt ggt ttc ctg ata gtc ctt gcc ctt ttt cag      843
Ser Ala Phe Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln
            225                 230                 235

gct ggg cta ggg aga atg atg atg aag tac aga gat cag aga gct ggg      891
Ala Gly Leu Gly Arg Met Met Met Lys Tyr Arg Asp Gln Arg Ala Gly
        240                 245                 250
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aag atc agt gaa aga ctt gtg att acc tca gaa atg att gaa aat atc      939
Lys Ile Ser Glu Arg Leu Val Ile Thr Ser Glu Met Ile Glu Asn Ile
    255                 260                 265

caa tct gtt aag gca tac tgc tgg gaa gaa gca atg gaa aaa atg att      987
Gln Ser Val Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile
270                 275                 280                 285

gaa aac tta aga caa aca gaa ctg aaa ctg act cgg aag gca gcc tat     1035
Glu Asn Leu Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr
                290                 295                 300

gtg aga tac ttc aat agc tca gcc ttc ttc ttc tca ggg ttc ttt gtg     1083
Val Arg Tyr Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val
            305                 310                 315

gtg ttt tta tct gtg ctt ccc tat gca cta atc aaa gga atc atc ctc     1131
Val Phe Leu Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu
        320                 325                 330

cgg aaa ata ttc acc acc atc tca ttc tgc att gtt ctg cgc atg gcg     1179
Arg Lys Ile Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala
    335                 340                 345

gtc act cgg caa ttt ccc tgg gct gta caa aca tgg tat gac tct ctt     1227
Val Thr Arg Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu
350                 355                 360                 365

gga gca ata aac aaa ata cag gat ttc tta caa aag caa gaa tat aag     1275
Gly Ala Ile Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys
                370                 375                 380

aca ttg gaa tat aac tta acg act aca gaa gta gtg atg gag aat gta     1323
Thr Leu Glu Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val
            385                 390                 395

aca gcc ttc tgg gag gag gga ttt ggg gaa tta ttt gag aaa gca aaa     1371
Thr Ala Phe Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys
        400                 405                 410

caa aac aat aac aat aga aaa act tct aat ggt gat gac agc ctc ttc     1419
Gln Asn Asn Asn Asn Arg Lys Thr Ser Asn Gly Asp Asp Ser Leu Phe
    415                 420                 425

ttc agt aat ttc tca ctt ctt ggt act cct gtc ctg aaa gat att aat     1467
Phe Ser Asn Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn
430                 435                 440                 445

ttc aag ata gaa aga gga cag ttg ttg gcg gtt gct gga tcc act gga     1515
Phe Lys Ile Glu Arg Gly Gln Leu Leu Ala Val Ala Gly Ser Thr Gly
                450                 455                 460

gca ggc aag act tca ctt cta atg atg att atg gga gaa ctg gag cct     1563
Ala Gly Lys Thr Ser Leu Leu Met Met Ile Met Gly Glu Leu Glu Pro
            465                 470                 475

tca gag ggt aaa att aag cac agt gga aga att tca ttc tgt tct cag     1611
Ser Glu Gly Lys Ile Lys His Ser Gly Arg Ile Ser Phe Cys Ser Gln
        480                 485                 490

ttt tcc tgg att atg cct ggc acc att aaa gaa aat atc atc ggt gtt     1659
Phe Ser Trp Ile Met Pro Gly Thr Ile Lys Glu Asn Ile Ile Gly Val
    495                 500                 505
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tcc tat gat gaa tat aga tac aga agc gtc atc aaa gca tgc caa cta     1707
Ser Tyr Asp Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu
510                 515                 520                 525

gaa gag gac atc tcc aag ttt gca gag aaa gac aat ata gtt ctt gga     1755
Glu Glu Asp Ile Ser Lys Phe Ala Glu Lys Asp Asn Ile Val Leu Gly
                530                 535                 540

gaa ggt gga atc aca ctg agt gga ggt caa cga gca aga att tct tta     1803
Glu Gly Gly Ile Thr Leu Ser Gly Gly Gln Arg Ala Arg Ile Ser Leu
            545                 550                 555

gca aga gca gta tac aaa gat gct gat ttg tat tta tta gac tct cct     1851
Ala Arg Ala Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro
        560                 565                 570

ttt gga tac cta gat gtt tta aca gaa aaa gaa ata ttt gaa agc tgt     1899
Phe Gly Tyr Leu Asp Val Leu Thr Glu Lys Glu Ile Phe Glu Ser Cys
    575                 580                 585

gtc tgt aaa ctg atg gct aac aaa act agg att ttg gtc act tct aaa     1947
Val Cys Lys Leu Met Ala Asn Lys Thr Arg Ile Leu Val Thr Ser Lys
590                 595                 600                 605

atg gaa cat tta aag aaa gct gac aaa ata tta att ttg aat gaa ggt     1995
Met Glu His Leu Lys Lys Ala Asp Lys Ile Leu Ile Leu Asn Glu Gly
                610                 615                 620

agc agc tat ttt tat ggg aca ttt tca gaa ctc caa aat cta cag cca     2043
Ser Ser Tyr Phe Tyr Gly Thr Phe Ser Glu Leu Gln Asn Leu Gln Pro
            625                 630                 635

gac ttt agc tca aaa ctc atg gga tgt gat tct ttc gac caa ttt agt     2091
Asp Phe Ser Ser Lys Leu Met Gly Cys Asp Ser Phe Asp Gln Phe Ser
        640                 645                 650

gca gaa aga aga aat tca atc cta act gag acc tta cac cgt ttc tca     2139
Ala Glu Arg Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser
    655                 660                 665

tta gaa gga gat gct cct gtc tcc tgg aca gaa aca aaa aaa caa tct     2187
Leu Glu Gly Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser
670                 675                 680                 685

ttt aaa cag act gga gag ttt ggg gaa aaa agg aag aat tct att ctc     2235
Phe Lys Gln Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu
                690                 695                 700

aat cca atc aac tct ata cga aaa ttt tcc att gtg caa aag act ccc     2283
Asn Pro Ile Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro
            705                 710                 715

tta caa atg aat ggc atc gaa gag gat tct gat gag cct tta gag aga     2331
Leu Gln Met Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg
        720                 725                 730

agg ctg tcc tta gta cca gat tct gag cag gga gag gcg ata ctg cct     2379
Arg Leu Ser Leu Val Pro Asp Ser Glu Gln Gly Glu Ala Ile Leu Pro
    735                 740                 745

cgc atc agc gtg atc agc act ggc ccc acg ctt cag gca cga agg agg     2427
Arg Ile Ser Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg
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750                 755                 760                 765

cag tct gtc ctg aac ctg atg aca cac tca gtt aac caa ggt cag aac     2475
Gln Ser Val Leu Asn Leu Met Thr His Ser Val Asn Gln Gly Gln Asn
                770                 775                 780

att cac cga aag aca aca gca tcc aca cga aaa gtg tca ctg gcc cct     2523
Ile His Arg Lys Thr Thr Ala Ser Thr Arg Lys Val Ser Leu Ala Pro
            785                 790                 795

cag gca aac ttg act gaa ctg gat ata tat tca aga agg tta tct caa     2571
Gln Ala Asn Leu Thr Glu Leu Asp Ile Tyr Ser Arg Arg Leu Ser Gln
        800                 805                 810

gaa act ggc ttg gaa ata agt gaa gaa att aac gaa gaa gac tta aag     2619
Glu Thr Gly Leu Glu Ile Ser Glu Glu Ile Asn Glu Glu Asp Leu Lys
    815                 820                 825

gag tgc ctt ttt gat gat atg gag agc ata cca gca gtg act aca tgg     2667
Glu Cys Leu Phe Asp Asp Met Glu Ser Ile Pro Ala Val Thr Thr Trp
830                 835                 840                 845

aac aca tac ctt cga tat att act gtc cac aag agc tta att ttt gtg     2715
Asn Thr Tyr Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val
                850                 855                 860

cta att tgg tgc tta gta att ttt ctg gca gag gtg gct gct tct ttg     2763
Leu Ile Trp Cys Leu Val Ile Phe Leu Ala Glu Val Ala Ala Ser Leu
            865                 870                 875

gtt gtg ctg tgg ctc ctt gga aac act cct ctt caa gac aaa ggg aat     2811
Val Val Leu Trp Leu Leu Gly Asn Thr Pro Leu Gln Asp Lys Gly Asn
        880                 885                 890

agt act cat agt aga aat aac agc tat gca gtg att atc acc agc acc     2859
Ser Thr His Ser Arg Asn Asn Ser Tyr Ala Val Ile Ile Thr Ser Thr
    895                 900                 905

agt tcg tat tat gtg ttt tac att tac gtg gga gta gcc gac act ttg     2907
Ser Ser Tyr Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu
910                 915                 920                 925

ctt gct atg gga ttc ttc aga ggt cta cca ctg gtg cat act cta atc     2955
Leu Ala Met Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile
                930                 935                 940

aca gtg tcg aaa att tta cac cac aaa atg tta cat tct gtt ctt caa     3003
Thr Val Ser Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln
            945                 950                 955

gca cct atg tca acc ctc aac acg ttg aaa gca ggt ggg att ctt aat     3051
Ala Pro Met Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn
        960                 965                 970

aga ttc tcc aaa gat ata gca att ttg gat gac ctt ctg cct ctt acc     3099
Arg Phe Ser Lys Asp Ile Ala Ile Leu Asp Asp Leu Leu Pro Leu Thr
    975                 980                 985

ata ttt gac ttc atc cag ttg tta tta att gtg  att gga gct ata gca    3147
Ile Phe Asp Phe Ile Gln Leu Leu Leu Ile Val  Ile Gly Ala Ile Ala
990                 995                 1000                1005

gtt gtc gca gtt tta  caa ccc tac atc ttt  gtt gca aca gtg cca       3192
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Val Val Ala Val Leu  Gln Pro Tyr Ile Phe  Val Ala Thr Val Pro
                1010                1015                1020

gtg ata gtg gct ttt  att atg ttg aga gca  tat ttc ctc caa acc      3237
Val Ile Val Ala Phe  Ile Met Leu Arg Ala  Tyr Phe Leu Gln Thr
                1025                1030                1035

tca cag caa ctc aaa  caa ctg gaa tct gaa  ggc agg agt cca att      3282
Ser Gln Gln Leu Lys  Gln Leu Glu Ser Glu  Gly Arg Ser Pro Ile
                1040                1045                1050

ttc act cat ctt gtt  aca agc tta aaa gga  cta tgg aca ctt cgt      3327
Phe Thr His Leu Val  Thr Ser Leu Lys Gly  Leu Trp Thr Leu Arg
                1055                1060                1065

gcc ttc gga cgg cag  cct tac ttt gaa act  ctg ttc cac aaa gct      3372
Ala Phe Gly Arg Gln  Pro Tyr Phe Glu Thr  Leu Phe His Lys Ala
                1070                1075                1080

ctg aat tta cat act  gcc aac tgg ttc ttg  tac ctg tca aca ctg      3417
Leu Asn Leu His Thr  Ala Asn Trp Phe Leu  Tyr Leu Ser Thr Leu
                1085                1090                1095

cgc tgg ttc caa atg  aga ata gaa atg att  ttt gtc atc ttc ttc      3462
Arg Trp Phe Gln Met  Arg Ile Glu Met Ile  Phe Val Ile Phe Phe
                1100                1105                1110

att gct gtt acc ttc  att tcc att tta aca  aca gga gaa gga gaa      3507
Ile Ala Val Thr Phe  Ile Ser Ile Leu Thr  Thr Gly Glu Gly Glu
                1115                1120                1125

gga aga gtt ggt att  atc ctg act tta gcc  atg aat atc atg agt      3552
Gly Arg Val Gly Ile  Ile Leu Thr Leu Ala  Met Asn Ile Met Ser
                1130                1135                1140

aca ttg cag tgg gct  gta aac tcc agc ata  gat gtg gat agc ttg      3597
Thr Leu Gln Trp Ala  Val Asn Ser Ser Ile  Asp Val Asp Ser Leu
                1145                1150                1155

atg cga tct gtg agc  cga gtc ttt aag ttc  att gac atg cca aca      3642
Met Arg Ser Val Ser  Arg Val Phe Lys Phe  Ile Asp Met Pro Thr
                1160                1165                1170

gaa ggt aaa cct acc  aag tca acc aaa cca  tac aag aat ggc caa      3687
Glu Gly Lys Pro Thr  Lys Ser Thr Lys Pro  Tyr Lys Asn Gly Gln
                1175                1180                1185

ctc tcg aaa gtt atg  att att gag aat tca  cac gtg aag aaa gat      3732
Leu Ser Lys Val Met  Ile Ile Glu Asn Ser  His Val Lys Lys Asp
                1190                1195                1200

gac atc tgg ccc tca  ggg ggc caa atg act  gtc aaa gat ctc aca      3777
Asp Ile Trp Pro Ser  Gly Gly Gln Met Thr  Val Lys Asp Leu Thr
                1205                1210                1215

gca aaa tac aca gaa  ggt gga aat gcc ata  tta gag aac att tcc      3822
Ala Lys Tyr Thr Glu  Gly Gly Asn Ala Ile  Leu Glu Asn Ile Ser
                1220                1225                1230

ttc tca ata agt cct  ggc cag agg gtg ggc  ctc ttg gga aga act      3867
Phe Ser Ile Ser Pro  Gly Gln Arg Val Gly  Leu Leu Gly Arg Thr
                1235                1240                1245
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gga tca ggg aag agt  act ttg tta tca gct  ttt ttg aga cta ctg       3912
Gly Ser Gly Lys Ser  Thr Leu Leu Ser Ala  Phe Leu Arg Leu Leu
                1250                1255                1260

aac act gaa gga gaa  atc cag atc gat ggt  gtg tct tgg gat tca       3957
Asn Thr Glu Gly Glu  Ile Gln Ile Asp Gly  Val Ser Trp Asp Ser
                1265                1270                1275

ata act ttg caa cag  tgg agg aaa gcc ttt  gga gtg ata cca cag       4002
Ile Thr Leu Gln Gln  Trp Arg Lys Ala Phe  Gly Val Ile Pro Gln
                1280                1285                1290

aaa gta ttt att ttt  tct gga aca ttt aga  aaa aac ttg gat ccc       4047
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Tyr Glu Gln Trp Ser  Asp Gln Glu Ile Trp  Lys Val Ala Asp Glu
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gtt ggg ctc aga tct  gtg ata gaa cag ttt  cct ggg aag ctt gac       4137
Val Gly Leu Arg Ser  Val Ile Glu Gln Phe  Pro Gly Lys Leu Asp
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ttt gtc ctt gtg gat  ggg ggc tgt gtc cta  agc cat ggc cac aag       4182
Phe Val Leu Val Asp  Gly Gly Cys Val Leu  Ser His Gly His Lys
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cag ttg atg tgc ttg  gct aga tct gtt ctc  agt aag gcg aag atc       4227
Gln Leu Met Cys Leu  Ala Arg Ser Val Leu  Ser Lys Ala Lys Ile
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ttg ctg ctt gat gaa  ccc agt gct cat ttg  gat cca gta aca tac       4272
Leu Leu Leu Asp Glu  Pro Ser Ala His Leu  Asp Pro Val Thr Tyr
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caa ata att aga aga  act cta aaa caa gca  ttt gct gat tgc aca       4317
Gln Ile Ile Arg Arg  Thr Leu Lys Gln Ala  Phe Ala Asp Cys Thr
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Val Ile Leu Cys Glu  His Arg Ile Glu Ala  Met Leu Glu Cys Gln
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Lys Cys Lys Ser Lys  Pro Gln Ile Ala Ala  Leu Lys Glu Glu Thr
                1460                1465                1470

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Glu Glu Glu Val Gln  Asp Thr Arg Leu
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atatcacttg tcaataaaat ccatacattt gtgt 6126

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<213> Cystic Fibrosis Transmembrane Conductor Regulator (CFTR) delta F508 mutation

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35 40 45

Leu Ser Glu Lys Leu Glu Arg Glu Trp Asp Arg Glu Leu Ala Ser Lys
50 55 60

Lys Asn Pro Lys Leu Ile Asn Ala Leu Arg Arg Cys Phe Phe Trp Arg
65 70 75 80

Phe Met Phe Tyr Gly Ile Phe Leu Tyr Leu Gly Glu Val Thr Lys Ala
85 90 95

Val Gln Pro Leu Leu Leu Gly Arg Ile Ile Ala Ser Tyr Asp Pro Asp
100 105 110

Asn Lys Glu Glu Arg Ser Ile Ala Ile Tyr Leu Gly Ile Gly Leu Cys
115 120 125

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Leu His His Ile Gly Met Gln Met Arg Ile Ala Met Phe Ser Leu Ile
145 150 155 160

Tyr Lys Lys Thr Leu Lys Leu Ser Ser Arg Val Leu Asp Lys Ile Ser
165 170 175

Ile Gly Gln Leu Val Ser Leu Leu Ser Asn Asn Leu Asn Lys Phe Asp
180 185 190

Glu Gly Leu Ala Leu Ala His Phe Val Trp Ile Ala Pro Leu Gln Val
195 200 205

Ala Leu Leu Met Gly Leu Ile Trp Glu Leu Leu Gln Ala Ser Ala Phe
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Cys Gly Leu Gly Phe Leu Ile Val Leu Ala Leu Phe Gln Ala Gly Leu
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Lys Ala Tyr Cys Trp Glu Glu Ala Met Glu Lys Met Ile Glu Asn Leu
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Arg Gln Thr Glu Leu Lys Leu Thr Arg Lys Ala Ala Tyr Val Arg Tyr
    290                 295                 300

Phe Asn Ser Ser Ala Phe Phe Phe Ser Gly Phe Phe Val Val Phe Leu
305                 310                 315                 320

Ser Val Leu Pro Tyr Ala Leu Ile Lys Gly Ile Ile Leu Arg Lys Ile
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Phe Thr Thr Ile Ser Phe Cys Ile Val Leu Arg Met Ala Val Thr Arg
            340                 345                 350

Gln Phe Pro Trp Ala Val Gln Thr Trp Tyr Asp Ser Leu Gly Ala Ile
        355                 360                 365

Asn Lys Ile Gln Asp Phe Leu Gln Lys Gln Glu Tyr Lys Thr Leu Glu
    370                 375                 380

Tyr Asn Leu Thr Thr Thr Glu Val Val Met Glu Asn Val Thr Ala Phe
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Trp Glu Glu Gly Phe Gly Glu Leu Phe Glu Lys Ala Lys Gln Asn Asn
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Phe Ser Leu Leu Gly Thr Pro Val Leu Lys Asp Ile Asn Phe Lys Ile
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Glu Tyr Arg Tyr Arg Ser Val Ile Lys Ala Cys Gln Leu Glu Glu Asp
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Val Tyr Lys Asp Ala Asp Leu Tyr Leu Leu Asp Ser Pro Phe Gly Tyr
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Arg Asn Ser Ile Leu Thr Glu Thr Leu His Arg Phe Ser Leu Glu Gly
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Asp Ala Pro Val Ser Trp Thr Glu Thr Lys Lys Gln Ser Phe Lys Gln
        675                 680                 685

Thr Gly Glu Phe Gly Glu Lys Arg Lys Asn Ser Ile Leu Asn Pro Ile
    690                 695                 700

Asn Ser Ile Arg Lys Phe Ser Ile Val Gln Lys Thr Pro Leu Gln Met
705                 710                 715                 720

Asn Gly Ile Glu Glu Asp Ser Asp Glu Pro Leu Glu Arg Arg Leu Ser
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Val Ile Ser Thr Gly Pro Thr Leu Gln Ala Arg Arg Arg Gln Ser Val
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Leu Arg Tyr Ile Thr Val His Lys Ser Leu Ile Phe Val Leu Ile Trp
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Tyr Val Phe Tyr Ile Tyr Val Gly Val Ala Asp Thr Leu Leu Ala Met
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Gly Phe Phe Arg Gly Leu Pro Leu Val His Thr Leu Ile Thr Val Ser
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Lys Ile Leu His His Lys Met Leu His Ser Val Leu Gln Ala Pro Met
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Ser Thr Leu Asn Thr Leu Lys Ala Gly Gly Ile Leu Asn Arg Phe Ser
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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17 February 2005 (17.02.2005)

PCT

(10) International Publication Number
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2003904237 8 August 2003 (08.08.2003) AU

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JAMES, David** [AU/AU]; 25 Cutler Road, Clontarf, New South Wales 2093 (AU). **GOVERS, Roland** [NL/NL]; Vioolstraat 25, NL-4702 CK Roosendaal (NL).

(74) Agent: **F. B. RICE & CO**; 605 Darling Street, Balmain, NSW 2041 (AU).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

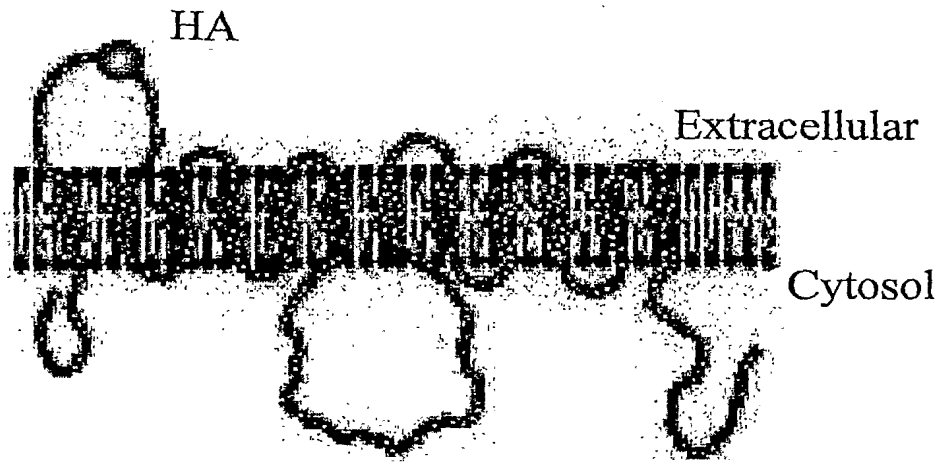
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
28 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL TRANSLOCATION ASSAY



(57) Abstract: The present invention relates to a novel in vitro assay for determining the level of a protein, in particular, a membrane transport protein that is located at the plasma membrane of a cell compared to the level of the protein in the cell. The process of the invention is also useful for determining the level of recycling of a membrane transport protein. The present invention additionally provides a process for identifying an agent that modulates the translocation of a protein, in particular, a membrane transport protein, to the plasma membrane and, as a consequence, the activity of that protein.

WO 2005/013666 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See "electronic data base" box below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See "electronic data base" box below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, CAPLUS, WPIDS: glut, glut1, glut4, traffic?, translocat?, tag, marker, ligand, bind, bound, receptor, lys?, disrupt?, permeabilis?, rupture?, assay, process, method, level

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 303 373 B1 (BOGAN et al.) 16 October 2001 Whole specification	1(in part), 2-62
P,A	US 6 632 924 B2 (BOGAN et al.) 14 October 2003 Whole specification	1(in part), 2-62
A	US 5 989 893 A (CZECH et al.) 23 November 1999 Whole specification	1(in part), 2-62
A	SLOT, J.W. et al., "Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rate", Proc. Natl. Acad. Sci. USA, September 1991, Vol. 88, pages 7815-7819 Whole article	1(in part), 2-62

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2005	21 FEB 2005
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **JAMIE TURNER** Telephone No : (02) 6283 2071

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HANEY, P.M., "Intracellular Targetting of the Insulin-regulatable Glucose Transporter (GLUT4) Is Isoform Specific and Independent of Cell Type", The Journal of Cell Biology, August 1991, Vol. 114, No. 4, pages 689-699 Whole article	1(in part), 2-62
X	WANG et al., "GLUT4 Translocation by Insulin in Intact Muscle Cells: Detection by a fast and Quantitative Assay", FEBS Letters, 1998, Vol. 427, pages 193-197 See especially paragraph 3.4 at page 195	1(in part), 2-62

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **1**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 1 is not limited to the technical features of the invention described in the international application. Clearly, there is support only for the determination of membrane transport proteins which are glucose transport (GLUT) proteins, notably GLUT1 and GLUT4. Because claim 1 is not limited to the detection of GLUT proteins it is not considered limited to the technical features of the invention.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2004/001057

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No:

It should be noted that an amended page 108, containing amendments to claims 53 and 54, was filed 5 October 2004 with the International Bureau. Unfortunately, because these amendments were not filed in the time frame referred to in Article 19 and Rule 46.1, these amendments cannot be considered under Art. 19. However, it should be noted that the subject matter of claims 53-54 (as if they were amended) was nevertheless the subject of the International Search Report. Further, it is apparent that the amendments to these pages do not go beyond the disclosure of the international application as filed.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2004/001057

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
US	6303373	AU	54775/00	EP	1189943	US	6632924
		US	2002052012	US	2002155479	WO	0075188
		WO	02059299				
US	5989893	AU	78448/94	EP	0721508	WO	9509240

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX